Adiponectin and Resistin as a non-invasive predictor for the severity of non-alcoholic fatty liver disease

Thesis

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Presented by

Ahmed Abd-Allah Salman
(M.B.B.Ch)

Supervised by

Prof. Dr. / SOHEIR A. ABO-ELFADL
Professor of Internal Medicine
Faculty of Medicine
Cairo University

Dr. / MONA A. HEGAZY
Assistant Professor of Internal Medicine
Faculty of Medicine
Cairo University

Dr. / LAILA A. RASHED
Assistant Professor of Medical Biochemistry
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University
2012

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Abstract: Fatty liver is a common condition increasingly detected by routine abdominal ultrasound.

The aim of the study is to investigate adiponectin and resistin as non-invasive predictors of NAFLD.

Subjects & Methods: Fifty four obese patients (BMI above 30), with suspected fatty liver by abdominal ultrasound were subjected to the following: Full history taking and physical examination, full anthropometric measurements, laboratory studies including serum adiponectin and resistin, abdominal US, and sonar-guided liver biopsy "for pathological examination and measuring adiponetin and resistin gene expression". Also fifteen age-matched healthy non-obese subjects were included as a control group for serum adiponectin & resistin. According to the results of biopsy, patients were subdivided into NASH group (46 patients) and non-NASH group (8 patients), and the 2 groups were compared as regards different parameters.

Results: Showed significantly lower levels of adiponectin & higher levels of resistin in NAFLD patients compared to control subjects. Also they showed lower levels of adiponectin & higher levels of resistin in the NASH group than the non-NASH group (but the difference was not significant). Serum AST, ALT, AAR, and GGT were higher in NASH than non-NASH group. Abdominal US showed a high sensitivity in the diagnosis of NAFLD.

Conclusion: Adiponectin and resistin can be combined in further studies with other noninvasive markers to predict the presence of NASH in order to replace liver biopsy.

Keyworsd: NAFLD, Obesity, Adiponectin, Resistin

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List of abbreviations

AAR	AST/ALT ratio.
ACO	Acetyl CO-A
ALP	Alkaline Phosphatase.
ALT	Alanine Aminotransferase.
AMPK	AMP-Activated Kinase.
ANGPTL3	Angiopoietin-like Protein 3
ApoBl00	Apolipoprotein B100.
APPL1	Adaptor Protein containing Pleckstrin homology domain.
APRI	Aspartate Aminotransferase to Platelet Ratio Index.
ARFI	Acoustic Radiation Force Impulse.
ASH	Alcoholic Steatohepatitis.
AST	Aspartate aminotransferase.
AUC	Area Under the Curve.
AUROC	Area Under the Receiver Operating Curve.
BMI	Body Mass Index.
C2CI4	Perchloroethylene.
CAP	Controlled Attenuation Parameter.
CCI4	Carbon tetrachloride.
CK-18	CytoKeratin-18.
CLD	Chronic Liver Disease.
CREBP	Carbohydrate Response Element Binding Protein.
CRP	C-Reactive Protein.
CT	Computed Tomography.
CVD	Cardiovascular disease.
DNA	Deoxy Ribonucleic Acid
DPI	Doppler Perfusion Index
ELF	Enhanced Liver Fibrosis.
ELISA	Enzyme Linked Immuno-Sorbent Assay.
ER	Endoplasmic Reticulum.
EtBr	Ethyl Bromide.
FBS	Fasting Blood Sugar.
FLI	Fatty Liver Index.
G6pase	Glucose-6-phosphatase.
Gck	Glucokinase.
GFR	Glomular Filteration Rate
GGT	Gamma Glutamyl Transferase.
HA	Hyaluronic Acid.

HAART	Highly Active Antiretroviral Drugs.
HBsAg	Hepatitis B Surface Antigen.
HCVAb	Hepatitis C Virus Antibody.
HDL-C	High Density Lipoprotein Cholesterol.
HMW	High Molecular Weight.
HOMA	Homeostasis Model Assessment .
HSCs	Hepatic Stellate Cells.
HU	Hounsefield Unit
ICAM-1	Intercellular Adhesion Molecule-1.
IL	Interleukin.
IU	International Unit.
IV	Intra Venous.
KDa	KiloDalton
Kg	Kilogram
LDL-C	Low Density Lipoprotein-Cholesterol.
LPS	LipoPolySaccharides.
MCP-1	Monocyte Chemoattractant Protein-1.
mL	Milliliter.
mm	Millimeter.
MRC	Mitochondrial Respiratory Chain.
MRI	Magnetic Resonance Imaging.
mRNA	Messenger Ribonucleic Acid.
MRS	Magnetic Resonance Spectoroscopy.
MS	Metabolic Syndrome.
NAFLD	Non Alcoholic Fatty Liver Disease.
NAS	NAFLD Activity Score.
NASH	Non Alcoholic Steatohepatitis.
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network.
NCEP ATP-III	National Cholesterol Education Program: Adult Treatment Program III
NEFA	Non-Esterified Fatty Acids.
NFS	NAFLD Fibrosis Score.
ng	Nanogram.
nm	Nanometer
NNFL	Non-NASH Fatty Liver.
NPV	Negative Predictive Value.
NT	Nash Test.
OELF	Original European Liver Fibrosis.
OSA	Obstructive Sleep Apnea.

P3NP	Procollagen III N-peptide.
PCR	Polymerase Chain Reaction.
PDGF	Platelet Derived Growth Factor.
PEPCK1	PhosphoEnolPyruvateCarboxyKinase 1.
pm	Picomole.
PPARs	Peroxisomal Proliferator Activated Receptors.
PPV	Positive Predictive Value.
PTX 3	Plasma Pentraxin 3.
qPCR	Quantitative Real Time PCR.
REC	Research Ethical Committee.
RNA	Ribonucleic Acid.
RNS	Reactive Nitrogen Species.
ROS	Reactive Oxygen Species.
RT-PCR	Reverse Transcriptase- Polymerase Chain Reaction.
SD	Standard Deviation.
SPEA	Serum prolidase enzyme activity.
sRAGE	Soluble Receptor for Advanced Glycation Endproducts.
SREBP	Sterol Regulatory Element Binding Protein.
ST	SteatoTest.
TG	Triglycerides.
TIMP 1	Tissue-Inhibited matrix Metalloproteinase Inhibitor-1.
TNF	Tumor Necrosis Factor.
TZD	Thiazolidinediones.
U/L	Unit/Liter.
UCP2	Uncoupling protein 2
UDCA	Ursodeoxycholic Acid.
ULN	Upper Limit of Normal.
US	UltraSonography.
USA	United States of America.
VCTE	Vibration Control Transient Elastography.
VEGF	Vascular Endothelial Growth Factor.
VLDL	Very Low-Density Lipoprotein
µL	Micro Liter.
µM	Micro Meter

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Introduction

Fatty liver is a common condition increasingly detected by routine abdominal ultrasound. Non- alcoholic steatohepatitis (NASH) is not rare and it is predicted to become one of the most common liver diseases **(Matteoni et al., 1999).** Liver biopsy represents the best diagnostic test for staging liver steatosis, inflammation and fibrosis, but medical and ethical considerations limit its use in subjects with non progressive fatty liver conditions **(Angelico et al., 2003).** Measuring serum adiponectin and resistin may serve as predictors of progressive liver pathology in NAFLD. Adiponectin is an insulin sensitizing adipokine possessing multiple beneficial effects on obesity-related medical complications and low level of this adipokine represents an independent risk factor for NAFLD **(Jiang et al., 2009).** Resistin is an adipocytokine whose physiologic role has been the subject of much controversy regarding its involvement with obesity and type 2 diabetes mellitus **(Steppan et al., 2001).**

Aim of the work

The aim of this work is to evaluate the use of serum adiponectin and resistin as a non invasive diagnostic test of fatty liver disease. This study will be conducted on 54 obese patients (BMI above 30kg/m^2) who will be subjected to: Full anthropometric measurements, full clinical examination, labs (FBS, total lipid profile, liver enzymes, HBsAg & HCVAb, serum adiponectin and resistin), abdominal ultrasound, and sonar-guided liver biopsy "for pathological examination and measuring adiponectin & resistin receptor gene expression". In addition we included 15 age-matched non-obese subjects as a control group to measure adiponectin & resitin serum level.

Chapter 1

(Anatomy and physiology of the liver)

The liver is among the most complex and important organs in the human body. Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system. A total loss of liver function leads to death within minutes, demonstrating the liver's importance **(Marieb., 2001).**

General description of the Liver:

The liver, the largest organ in the body, weighs 1200- 1500 gm and comprises one-fiftieth of the total adult body weight. It is relatively larger in infancy, comprising one eighteenth of the birth weight. This mainly due to a large left lobe. It occupies a large region mostly on the right side of the body, below the diaphragm and behind the ribs 5 through 10 **(Leftkowitch., 2011).**

The liver has many functions, primarily including:

• Acting as a gatekeeper between the digestive system and the circulatory system.

• Processing toxic substances before they enter general circulation.

• Storing and converting nutrients for future use.

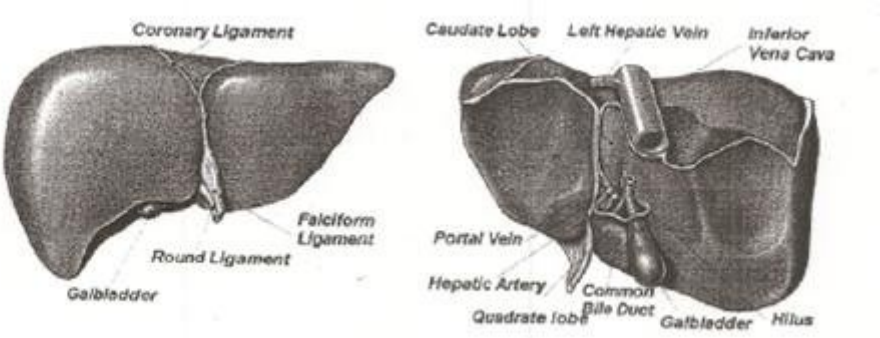
• Synthesizing most plasma proteins.

• Secreting bile into small intestine to break down fats **(Marieb., 2001).**

Gross Anatomy:

The liver is divided into four lobes which are right, left, caudate, and quadrate. The right and left lobes are the largest, while the caudate and

quadrate are smaller and located posteriorly. Superiorly, the falciform ligament separates the right and left lobes. Inferior to the falciform ligament is the round ligament, which protrudes from the liver slightly. Also visible anteriorly on the most inferior portion of the right lobe is the gall bladder. The caudate lobe is located superiorly, approximately between the right and left lobes. Adjacent to the caudate lobe is the sulcus for the inferior vena cava. Just inferior to the caudate lobe is the porta hepatis, where the hepatic artery and hepatic portal vein enter the liver. The portal vein carries nutrient laden blood from the digestive system. Inferior to the porta hepatis is the bile duct which leads back to the gallbladder. Finally, the hepatic vein, where post-processed blood leaves the liver, is found inferior and adjacent to the sulcus for the inferior vena cava. The liver is held on place by a system of mesenteries posteriorly, and is also attached to the diaphragm via the falciform ligament. Additionally, most of the liver is covered by visceral peritoneum **(Heuman., 1997).**



(Figure 1-1): Anatomy of the liver (Heuman., 1997).

Microscopic anatomy:

The basic functional unit of the liver is the liver lobule. A single lobule is about the size of a sesame seed and is roughly hexagonal in shape. The primary structures in a lobule include:

- Plates of hepatocytes form the bulk of the lobule

- Portal triads at each corner of hexagon
- Central vein
- Liver sinusoids that run from the central vein to the portal triads
- Hepatic macrophages (Kupffer cells)
- Bile canaliculi ("little canals") - formed between walls of adjacent hepatocytes
- Space of Disse - a small space between the sinusoids and the hepatocytes

The portal triads consist of three vessels: a hepatic portal arteriole, hepatic portal venule, and a bile duct. The blood from the arteriole and the venule both flow in the same direction through the sinusoids toward the central vein, which eventually leads to the hepatic vein and the inferior vena cava. Secreted bile flows in the opposite direction through the bile canaliculi away from the central vein, toward the portal triad, and exiting via the bile duct. As blood flows through the sinusoids and the space of Disse toward the central vein, nutrients are processed and stored by the hepatocytes. Moreover, worn out blood cells and bacteria are engulfed by the Kupffer cells **(Stevens et al., 1997).**

Interrelationships with other organs:

The liver interacts with many other organs.

- Following the flow of blood, the liver receives its arterial blood supply from the hepatic arteries. The hepatic arteries originate from abdominal aorta distal to the celiac trunk. Thus the liver receives its oxygenated blood supply from the heart.
- Nutrient laden blood from the digestive system and blood leaving the spleen enters the liver through the hepatic portal vein. Processed blood leaving the liver through the hepatic