A study of Interleukin 6 as a predictive biomarker for development of Nonalcholic steatohepatitis in patients with Nonalcholic fatty liver disease

Thesis

Submitted for partial fulfillment of Master Degree
Of Internal Medicine

By

Maha Aboulfotouh Sayed
M.B.B.C.H Faculty of Medicine Ain shams University

Under Supervision of

Prof.Dr. khaled Abdel Hamed Mohamed
Professor of internal medicine, Gastroenterology and Hepatology
Faculty of Medicine Ain shams University

Asst.prof.Dr. Enas El Khadr Mohamed
Assistant Professor of internal medicine, Gastroenterology and Hepatology
Faculty of Medicine Ain shams University.

Dr. Dina Morsy Ahmed
Lecturer of internal medicine, Gastroenterology and Hepatology.
Faculty of Medicine Ain shams University.

Faculty of Medicine
Ain Shams University
2018

سورة البقرة الآية: ٣٢

Acknowledgement

First and foremost, all thanks and gratitude to *Allah*, the Most Merciful, who gives us the power to accomplish this work.

I would like to express my deepest gratitude and cordial appreciation to ***Prof.Dr. khaled Abdel Hamed Mohamed*****;** Professor of Gastroenterology and Hepatology Faculty of Medicine Ain shams University, for his expert advices and meticulous guidance and support throughout this work.

I feel deeply indebted to ***Asst.prof.Dr. Enas El Khadr Mohamed****;* Assistant Professor of Gastroenterology and Hepatology, Faculty of Medicine Ain shams University, who gave me much of the efforts, time, experience and close supervision throughout the work. They provided me with continuous encouragement and assistance throughout this work.

I`m also deeply grateful for *all patients* included in this study for being cooperative.

Maha Aboulfotouh Sayed

LIST OF CONTENTS

LIST OF ABBREVIATIONS

Abb.		Full Term
ALP	:	Alkaline phosphatase
ASAP	:	AST to Platelet Ratio Index
AST	:	Aspartate aminotransferase
ATGL	:	Adipocyte triclyceride lipase
ATP	:	Adenosine triphosphate
AUROC	:	The Area Under an ROC Curve
BCAA	:	Branched chain amino acids
BDNF	:	brain-derived neurotrophic factor
BMI	:	Body mass index
Ca2+	:	Calcium
CBC	:	Complete blood count
CD	:	Cluster of Differentiation
CHF	:	Congestive heart failure
CHOL	:	Cholesterol
CK-18	:	Cytoke ratin-18
CLC	:	cardiotrophin-like cytokine
CNTF	:	ciliary neurotrophic factor
CRP	:	C-reactive protein
CT	:	Computed Tomography
CT-1	:	cardiotrophin-1
CYP 2E1	:	Cytochrome P-450 2E1
DNMT1	:	DNA methyltransferase-1
DPI	:	Doppler perfusion index
ELF	:	Enhanced Liver Fibrosis
ELISA	:	Enzyme linked immunosorbent assays
EMA	:	European Medicines Agency
EV71	:	Enterovirus 71
FBS	:	Fasting blood sugar
FFAs	:	Free fatty acids
FH	:	Familial hypercholesterolaemia
GGT	:	Gamma-glutamyl transferase
Gp130	:	Glycoprotein130
GSH-PX	:	Glutathione peroxidase
Hb	:	Hemoglobin

LIST OF ABBREVIATIONS

Abb.		Full Term
HbA1C	:	Hemoglobin A1C (Glycated Hb)
HBsAg	:	Hepatitis B surface antigen
HCC	:	Hepatocellular carcinoma
HCV	:	Hepatitis C virus
HDAC1	:	Histone Deacetylase 1
HDL	:	High-density lipoprotein
HMG CO-A	:	Hydroxyl-3-methylglutaryl coenzyme A
HTGC	:	Hepatic triglyceride content
HTN	:	Hypertention
Hus	:	Hounsfield units
IL-1ra	:	IL-1 receptor antagonist
IL6ST	:	IL-6 signal transducer
ILs	:	Interleukins
INR	:	International normalized ratio
IRAS	:	Insulin Resistance Atherosclerosis Study
JAKs	:	Janus kinases
KSHV-IL6	:	Kaposi's sarcoma-associated herpes virus interleukin 6-like protein
LDL	:	Low-density lipoprotein
LDL-C	:	Low-density lipoprotein cholesterol
LIF	:	leukemia inhibitory factor
LPL	:	Lipoprotein lipase
LSM	:	Liver stiffness measurement
MAPK	:	mitogen-activated protein kinase
MRI	:	Magnetic Resonance Imaging
MRS	:	Magnetic Resonance Spectroscopy
MS, MetS	:	Metabolic syndrome
NAFL	:	Non alcoholic fatty liver
NAFLD	:	Non alcoholic fatty liver disease
NAS	:	NAFLD Activity Score
NASH	:	Non alcoholic steatohepatitis
NFAT	:	nuclear factor of activated T-cells
NFκB	:	Nuclear factor-κB
NPC	:	Niemann-Pick type C protein

LIST OF ABBREVIATIONS

Abb.		Full Term
OSM	:	oncostatin M
P62	:	Nucleoporin p62
PAMPs	:	pathogen-associated molecular patterns
PGE	:	Prostaglandin E
PI3K	:	Phosphoinositide 3-kinase
PIIINP	:	Procollagen III N-Terminal Propeptide
PKB	:	protein kinase B
Plt	:	Platelets
PPAR-γ	:	peroxisome proliferator activator receptor gamma
PRRs	:	pattern recognition receptors
PT	:	prothrombin time
PTX3	:	Plasma pentraxin 3
PUFA	:	Polyunsaturated fatty acids
RCT	:	Randomized control trial
ROS	:	Reactive oxygen species
SCOUT	:	The Sibutramine Cardiovascular Outcome
sIL-6R	:	soluble form of IL-6Receptor
SPEA	:	Serum prolidase enzyme activity
SPSS	:	Statistical Package for Social Sciences
STATs	:	Signal Transducers and Activators of Transcription
T II DM	:	Type 2 diabetes mellitus
TGF	:	Transforming Growth Factor
TIMP	:	Tissue inhibitor of metalloproteinases
Tlc	:	Total leucocytic count
TLRs	:	Toll-like receptors
TNF	:	Tumor necrosis factor
TRX	:	Thioredoxin
TZD	:	Thiazolidinedione
U/S	:	Ultrasonography
WAT	:	White adipose tissue

LIST OF TABLES

LIST OF FIGURES

Figure No.	Title	Page No.

A study of Interleukin 6 as a predictive biomarker for development of nonalcholic steatohepatitis in patients with Nonalcholic fatty liver disease

Thesis

Submitted for partial fulfillment of Master Degree of Internal Medicine.

By

Dr. Maha Aboulfotouh Sayed

M.B.B.C.H Faculty of Medicine Ain shams University.

Under supervision of

Prof.Dr. khaled Abdel Hamed Mohamed

Professor of Internal Medicine, Gastroenterology and Hepatology

Faculty of Medicine Ain shams University.

Asst.prof.Dr. Enas El Khadr Mohamed

Assistant Professor of Internal Medicine, Gastroenterology and Hepatology

Faculty of Medicine Ain shams University.

Dr. Dina Morsy Ahmed

Lecturer of Internal Medicine, Gastroenterology and Hepatology.

Faculty of Medicine Ain shams University.

Introduction

The spectrum of NAFLD is a continuum ranging from simple steatosis to NASH and finally cirrhosis.

The defining characteristic of the disease is the presence of greater than normal lipid deposition within the liver with the absence of excessive alcohol consumption defined as > 20 g/d for men and 10 g/d for women.

Steatosis is the presence of lipid within the cytoplasm of hepatocytes, the criteria for which is defined in the literature as being either hepatic lipid levels above the 95th percentile for healthy individuals (about >55 mg/g liver) greater than 5% of the liver's weight or found in greater than 5% of hepatocytes histologically.

NASH is defined as steatosis in the presence of hepatocyte damage, inflammation and/or subsequent scarring and replacement of the tissue with type I collagen. **(Hassan et al., 2014)**

The development of NASH is considered to be through a ''two hit'' process. The first "hit" includes accumulation of fat in liver cells, the second "hit" causes hepatocyte inflammation and necrosis which lead to cirrhosis and fibrosis. **(Papandreou et al., 2007)**

Since the term NASH was first coined by Ludwig et al. in 1980 the prevalence of NAFLD has risen rapidly in parallel with the dramatic rise in population levels of obesity and diabetes, NAFLD now representing the most common cause of liver disease in the Western world.

The prevalence of NAFLD is estimated to be between 20% and 30% in Western adults, rising to 90% in the morbidly obese. NASH, the more advanced and clinically important form of NAFLD is less common, with an estimated prevalence of 2–3% in the general population16 and 37% in the morbidly obese. **(Dowman et al., 2011)**

Most subjects with NAFLD are clinically silent and asymptomatic. Ultrasonography still represents the first-line diagnostic tool for simple liver steatosis.

(Wong et al., 2010)

Liver biopsy is still the gold standard in diagnosis of NASH. **(Abdel kader and Ashmawy, 2015)**

Knowledge of whether a patient has simple steatosis or NASH is very important prognostically.

With increasing liver fibrosis the ALT typically falls and the AST remains stable or rises.

Staging fibrosis is essential in all patients with NAFLD to identify subjects with advanced fibrosis . **(Dyson et al., 2013)**

The NAFLD fibrosis score (NFS) is a panel comprising six variables of age, hyperglycaemia, BMI, platelet count, albumin and AST/ALT ratio, which was constructed using a large panel of 733 biopsy-proven NAFLD patients across several centres worldwide. **(J k et al., 2010)**

−1.675+0.037×age (years)+0.094×BMI (kg/m2)+1.13×IFG or diabetes (yes=1, no=0)+0.99×AST/ALT ratio—0.013×platelet (×109/L)—0.66×albumin (g/dL)

Advanced fibrosis can be reliably excluded (NPV 93%) using the low cut-off score (<−1.455) and diagnosed with high accuracy (PPV 90%) using the high cut-off score (>0.676) . **(Dyson et al., 2013)**

IL-6 is a proinflammatory pleiotropic cytokine produced by adipocytes, hepatocytes, immune and endothelial cells

Wieckowska et al.demonstrated a markedly increased IL-6 expression in liver tissue of patients with NASH as compared to simple steatosis or normal liver. **(Sanja et al., 2014)**

Aim of work

To predict nonalcholic steatohepatitis in patient with NAFLD through measurement of interleukin 6 to predict progression of the disease into liver cirrhosis through early diagnosis .

Methods and patients

Type of study: **Case control study**

Patients will be selected from El Demerdash Hospital outpatient clinic of liver.

We will select:

- 40 cases of simple steatosis (u/s showing changes of fatty liver and ALT, AST within normal ranges with no advanced fibrosis by NAFLD fibrosis score)
- 30 patients of NASH (u/s showing changes of fatty liver with elevated ALT, AST)
- 20 healthy control with normal u/s and normal ALT, AST .

Exclusion criteria

1. Liver cirrhosis by u/s.
2. Alcoholic
3. Hepatitis B or C positive .

4. Any cause of elevated liver enzymes as Wilson disease ,Autoimmune hepatitis Hemochromatosis ,drugs etc..

5.BMI >40

All cases and controls will be tested for interleukin 6 using an enzyme-linked immunosorbent assay kit after taking an informed consent.

This thesis will include:

- Introduction
- aim of the work.
- Review of literature .
- Subject and methods .
- Results.
- Discussion.
- Summary and conclusion .
- References .
- Arabic summary.

References

1. Kareem Hassan, Varun Bhalla, Mohamed Ezz El Regal et al. (2014): nonalcoholic fatty liver disease: A comprehensive review of a growing epidemic. J Gastroenterol Hepatol 12082-
12101

2. Dowman JK, Tomlinson JW and Newsome PN. (2011): Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis; 33(5):525-40.

3.Angulo P and Lindor KD. (2002): Non-alcoholic fatty liver disease. J Gastroenterol Hepatol.; 17 Suppl: S186–S190

4. Papandreou D, Rousso I and Mavromichalis I. (2007): Update on non-alcoholic fatty liver disease in children. Clin Nutr. ; 26:409–415

5. Shehab M Abd El-Kader and Eman M Salah El-Den Ashmawy. (2015): Non-alcholic fatty liver disease: The diagnosis and management World J Hepatol.; 7(6): 846–858.

6.Wong VW, Vergniol J, Wong GL, et al. (2010): Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010; 51:454–462.

7.Sanja Stojsavljević, Marija Gomerčić Palčić, Lucija Virović Jukić, et al.(2014): Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease .World J Gastroenterol. 2014 Dec 28; 20(48): 18070–18091.

8.Jessica K Dyson, Quentin M Anstee, and Stuart McPherso. (2013):
Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. Frontline Gastroenterol. 2014 Jul; 5(3): 211–218.