CORE ANTIGEN LEVELS AND MTHFR POLYMORPHISM AS MARKERS FOR LIVER STEATOSIS IN CHRONIC HCV GENOTYPE 4 PATIENTS

By

EMAN MOHAMED MAHMOUD MOHAMED
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006

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APPROVAL SHEET

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APPROVAL COMMITTEE

Dr. YAHYA SHAKER MOHAMED HAMED……..……...
Researcher Professor of Biochemistry, National Research Centre

Dr. MOHI El DDIN ALI OSMAN…….…....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. AHMED ABO ABOUL-ENEIN……..…...
Professor of Biochemistry Fac. Agric., Cairo University

Dr. SAYED ABD EL-KADER SAYED……..……...…..
Professor of Biochemistry Fac. Agric., Cairo University

Date: 22 / 04 / 2017

SUPERVISION SHEET

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Dr. AHMED MAHMOUD ABOUL- ENEIN
Professor of Biochemistry Fac. Agric., Cairo University

Dr. SAYED ABD EL-KADER SAYED
Professor of Biochemistry Fac. Agric., Cairo University

Dr. MOSTAFA KAMEL EL-AWADY
Researcher Professor of molecular genetics, National Research Center

Name of Candidate: Eman Mohamed Mahmoud **Degree:** M.Sc.

Title of Thesis: Core antigen levels and MTHFR polymorphism as markers for liver Steatosis in chronic HCV genotype 4 patients.

Supervisors: Dr. Ahmed Mahmoud Aboul-Enein
Dr.Sayed Abd-Elkader Sayed
Dr.Mostafa Kamel El-Awady

Department: Biochemistry **Approval:** 22 / 4 / 2017

ABSTRACT

Methylene tetrahydrofolate reductase (MTHFR) 677CT polymorphism was reported as a genetic variant in liver steatosis and fibrosis.This research was conducted to study the association between MTHFR 677CT polymorphism and HCV core with severity of steatosis in HCV GT4 patients. 111 HCV patients and 112 control subjects were recruited. Polymorphism was detected by RFLP analysis, core Ag was detected by ELISA. The study included 56 (51%) males and 55 (49%) females. The median age of the recruited patients was 39.4±8.6 years. The mean of (BMI) was 26.6±2.38 kg/m^2. The degree of steatosis was correlated with age (*p*-value< 0.018), BMI (*p*-value< 0.003), platelets (*p*-value< 0.001), albumin (*p*-value< 0.001), and Hb (*p*-value< 0.023) But there were no statistically significant differences between different grades of steatosis and serum ALT, AST, WBCs, Bilirubin, HCV RNA concentration and gender. Further statistical analysis showed a strong correlation between steatosis and the progression rate of fibrosis. The frequencies of MTHFR 677C→T genotypes (CC, CT, and TT) among chronic HCV patients who have steatosis were 32%, 50%, and 18%, respectively among controls where 65%, 28%, and 7% respectively, whereas among patients who have no steatosis were 63%, 33%, and 4% respectively. Comparing HCV patients with steatosis to controls revealed that the risk steatosis was 3.63 fold higher in patients who have steatosis the than in patients without the T substitution (95% CI 1.92-6.82) and 5.21 fold in patients with two alleles (95% CI 2.01-13.54) . When comparing the chronic HCV patients with steatosis to those without steatosis, the data showed that patients with single allele substitution i.e. CT had 2.88 fold more risk to develop steatosis than those having CC genotype (95% CI 1.08-7.68) . While those with 2 allele subsititution i.e. TT are 8.57 fold higher risk to develop steatosis (95% CI 1.03-71.08). The normal (C) allele frequency of MTHFR at position 677 in 87 chronic HCV patients who have steatosis was 57% while in controls it was 79%. The mutant (T) allele frequency in 87 chronic HCV patients who have steatosis was 43% while in controls it was 21% . We investigated the level of the circulating HCV core protein in the recruited patients. The data illustrate that there is no significant difference between the core antigen titer and degree of steatosis.

Key words: HCV; MTHFR C677T polymorphism; liver steatosis

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my soul daughter Sara and Lara, as well as to my husband Aseem and my Mother for all the support their lovely offered along the period of my post graduation.

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ABBREVIATIONS

A	Adenine
AdoMet	S-adenosyl methionine
AdoHcy	Adenosyl homocystine
ADRP	Adipocyte differentiation-releated protein
AICAR	5-amino-4-imidazolecarboxamide ribonucleotide transferase
APS	Amonium per sulphate
ATP	Adenosine triphosphate
AcLDL	Acylated LDL AcLDL
ALT	Alanine aminotransferase
AOX	Acyl coA oxidase
AST	Aspartat aminotransferase
BHMT	Betaine homocysteine methyl transferase
bp	Base pair
BMI	Body mass index
BSA	Bovine serium albumin
CPT-1	Carnitine palmitory transferase-1
C	Cytosine
CBS	Cystathionine β-synthase
CI	Confidence interval
dATP	Deoxy adenine triphosphate
dCTP	Deoxy cytosine triphophate
dGTP	Deoxy guanine triphosphate
DGAT1	Diacylglycerol Acyltransferase 1
DHF	Dihydrofolate.
DHFR	Dihydrofolate reductase.
DNA	Deoxy nucleic acid
dNTPs	Deoxy nucleotides triphophate
DS	Down syndrome
dTMP	Deoxy thymidine monophosphate
dUMP	Deoxy uridine monophosphate
dUTP	Deoxy uridine triphosphate
EDTA	Ethylene diamine tetra acetic acid
ER	Endoplasmic reticulum
FAD	Flavine adenine dinucleotide
FGAR	Formyl glycinamide ribonucleotide

G	Guanine
GAR	Glycinamide ribonucleotide transformylase
HB	haemoglobin
HcG	Human chorionic gonadotrophins
HCV	Hepatitis C Virus
HCC	Hepatocellular carcinoma
HDL	High density lipoprotein
Kb	Kilo base
KDa	Kilo Dalton.
LDLr	Lipoprotein receptor.
LDs	Lipid drops
LDLR	Lipoprotein receptor
MAT	Methionine adenosyl transferase
MeTHF	Methyl tetrahydrofolate
Mol	Mole
MS	Methionine synthase
MSAFB	Maternal alpha-feto protein
MT	Methyl transferase
MTHF	Methylene tetrahydrofolate
MTHFR	Methylene tetrahydrofolate reductase
MTRR	Methionine synthase reductase
mRNA	Messenger RNA
NADP	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non- alcoholic fatty liver disease
nM	Nano mole
NRC	National research centre
NTD	Neural tube defect.
NTRs	Non –translated regions
O.D	Optical density
ORF	Open reading frame
OR	Odds ratio
PCR	Polymerase chain reaction
Pte Glu	Pteroyl glutamic acid = folic acid
P-value	Significance value
PEG-IFNα	Pegylated interferon alfa
RFLP	Restriction fragment length polymorphism
PPARα	Peroxisome proliferator-activated receptor
PKR	Protein kinase function
RNA	Ribo nucleic acid

SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine
SHMT	Serine hydroxyl methyl transferase
SVR	Sustained virological response
SNPs	Single nucleotide polymorphisms
ssRNA	Sense single-stranded
SREBP-1C	Sterol regulatory element binding protein -1c
T	Thiamine
TG	Triglyceride
TEMED	N,N,N,N-Tetramethylethylene diamine.
THF	Tetrahydrofolate
vLDL	Very low density lipoprotein
X^2	Chi-square

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INTRODUCTION

Chronic infection with the hepatitis C virus (HCV) is a leading cause of global morbidity and mortality (McGowan, Monis *et al.,* 2013).

Hepatic steatosis refers to excessive fat accumulation in the liver, which is very common in the general population and also in patients infected with hepatitis C virus (HCV) and contributes to the chronic hepatitis and progressive hepatic injury that can lead to end-stage liver disease and hepatocellular carcinoma (Dev *et al.,* 2004 and National Center for HIV/AIDS 2006) This is more frequently in patients infected by genotype 3 (Mihm *et al.,* 1997and Patton *et al.,* 2004). HCV is responsible for approximately 50 % prevalence of steatosis among patients undergoing a liver biopsy (Fiore *et al.,* 1996 and Patton, 2004).

Both host and viral factors have been demonstrated to play an important role in the development of steatosis. Patients infected with HCV genotype 3 have steatosis that correlates with serum HCV RNA levels, resolves with successful therapy and is independent of host factors (Ramalho *et al.*, 2013).

Genotype 3–infected patients have steatosis that is more frequent and severe than genotype 1–infected patients (Hezode et *al.,* 2004). Despite these findings, not all patients with genotype 3 infection have steatosis. These observations support a 2-pathway model of steatosis formation: one involving viral factors present in most genotype 3 isolates and absent or reduced in other genotypes and another relying on manipulation