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The Role of MicroRNA in Hepatitis C Infection

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I declare that this thesis has been composed by myself and that the work which is recorded here has been done by myself. It has not been submitted for a degree at this or any other university.

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Dedication

I dedicate this work to my father, mother, brother, husband, friends and those from whom I have learned, whenever and wherever they are.

Fatma El-Zahraa Mohamed

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ABSTRACT

Background: Hepatitis C virus (HCV) infection is the major cause for chronic hepatitis. Interferons (IFNs) are a large family of proteins involved in antiviral defense and used for HCV treatment. Large numbers of Egyptian patients are not responding to the combined pegylated interferon alpha /ribavirin therapy. MicroRNAs (miRNAs) are class of post transcription regulators that play important role in HCV life cycle and interfering with interferon signaling or production.

Aim: To find a predictive measure for the response to the combined pegylated interferon alpha /ribavirin therapy using miR-20a, miR-155, miR-21 and miR-196b with evaluating MxA-mRNA expression levels as a positive control for interferon action.

Methods: This study was conducted on 100 patients, 50 responders and 50 non-responders with HCV and 20 healthy controls. MiRNAs and MxA-mRNA were isolated from serum and blood sequentially and the expression levels were quantified using quantitative PCR (qPCR).

Results: All the studied miRNAs; miR-20a, miR-155, miR-21 and miR-196b were up regulated in non-responders compared to responders patients. These data were confirmed by the inhibition of MxA-mRNA levels in non-responders compared to responders patients. Receiver operator characteristic (ROC) analysis revealed that the cut-off value for responding to the combined pegylated interferon alpha /ribavirin therapy for miR-20a, miR-155, miR-21 and miR-196b is >1, >2.26, >1.57 and >2.49 respectively.

Conclusion: The circulating miRNAs; miR-20a, miR-155, miR-21 and miR-196b in serum are promising biomarkers for predicting the responding HCV-4 Egyptian patients to the combined pegylated interferon alpha /ribavirin therapy.

LIST OF ABBREVIATIONS

A	Adenosine
ADARs	Adenosine deaminases acting on RNA
Ago2	Argonaute-2
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under curve
bps	Base pairs
C.elegens	*Caenorhabditis elegens*
cal	Calibrators
cDNA	Complementary DNA
CHC	Chronic hepatitis C
Ct	Cycle threshold
D. melanogaster	*Drosophila melanogaster*
DGCR8	DiGeorge critical region 8
dNTPs	Deoxynucleotide triphosphate
dsDNA	Double stranded DNA

dT	Deoxy- thymine nucleotide
dTTP	Deoxythymidine triphosphate
EDTA	Ethylenediaminetetraacetic acid
FA	Fanconi Anemia
FADD	Fas associated protein with death domain
FRET	Fluorescent resonance energy transfer
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GTPases	Guanosine triphosphatase
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCV-4	HCV genotype 4
HIV	Human immunodeficiency virus
HMOX1	Heme oxygenase 1
Huh7	Human hepatocarcinoma cell line
IC	internal control
IFITM1	Interferon induced transmembrane protein 1
IFNs	Interferons

IFN-α	Interferon alpha
IFN-β	Interferon beta
IFN-γ	Interferon gamma
IFN-λ	Interferon lambda
IGF2BP1	Insulin like growth factor 2 mRNA binding protein 1
IL	Interleukin
IRAK1	Interleukin-1 receptor-associated kinase 1
IRF-1	Interferon regulatory factor 1
IRF-2	Interferon regulatory factor 2
ISGF3	IFN-stimulated gene factor 3
ISGs	Interferon stimulated genes
ISRE	IFN-stimulated response element
MIR155HG	MiR-155 host gene
miRNAs	MicroRNAs
MxA	Myxovirus resistance A
MyD88	Myeloid differentiation primary response gene 88
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells

NK	Natural killer
NS	Non-structural
NS5A	Nonstructural 5A protein coding region
nt	Nucleotide
PACT	protein activator of PKR
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
Peg- IFN-α	Pegylated interferon alpha
PEG-IFN-RBV	Pegylated interferon alpha plus ribavirin
Pre-miRNA	Precursor miRNA
Pri-miRNA	Primary miRNA transcripts
qPCR	Quantitative polymerase chain reaction
RIP	Receptor interacting protein
RISC	RNA induced silencing complex
RLC	RISC loading complex
RNase	Ribonuclease
ROC	Receiver operating characteristic
RT	Reverse transcription

RT-PCR	Reverse transcription PCR
RT-qPCR	Quantitative reverse transcription PCR
Tth	Thermostable polymerase enzyme
RVR	Rapid virological response
siRNA	Small interfering RNA
Smad7	Mothers against Decapentaplegic homolog 7
snoRNA	Small nucleolar RNA
SOCS1	Suppressor of Cytokine Signaling 1
ssDNA	Single stranded DNA
STDF	Science and technology development fund in Egypt
SVR	Sustained virological response
TBRI	Theodor Bilharz Research institute
TGF-β	Transforming growth factor beta
TLRs	Toll-like receptors
Tm	Melting temperature
TMEM49	Transmembrane protein 49
TNFα	Tumor necrosis factor alpha

TRBP	Tar RNA binding protein
TU	Transcription unit
UNG	Uracil N-glycosylase
USP18	Ubiquitin Specific Peptidase 18
UTRs	Untranslated regions

LIST OF FIGURES