

# **“In Vitro Inhibition of HCV Replication”**

A thesis Submitted to  
Biochemistry Department  
Faculty of Science  
Ain Shams University

**For the degree of Doctor of Philosophy  
in Science (in Biochemistry).**

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2005**

## **Abstract**

The outcome of interferone plus ribavirine treatment of hepatitis C virus (HCV) genotype 4 is unfortunately poor. Development of alternative therapy for this genotype is of a paramount importance. Inhibitions of HCV gene expression in vitro by the use of antisense phosphorothioate oligodeoxynucleotides (S-ODN) against (IRES) elements were associated with favorable results. To assess S-ODN activity, previous studies utilized viral subgenomic or full cDNA fragments linked to reporter genes transfected into adhered cells or in a cell free system. In the present study we utilized HepG2 cells infected with native HCV RNA genomes in a replication competent system. S-ODN against stem loop IIIId (S-ODN2, nt 252-270) and the AUG translation start site (S-ODN1, nt 325-348) of the viral polyprotein precursor were used as potential inhibitors for viral replication. Intracellular viral replication was monitored both by nested RT-PCR and real time PCR technology. These experiments indicated that intracellular replication of HCV genotype 4 was completely arrested by using either S-ODN structure (with more efficacy of S-ODN1 than S-ODN2) at concentrations as low as 1  $\mu$ M after 48 h. in culture. The inhibitory effect of S-ODN appeared to be specific to HCV replication in light of the consistent levels of human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression throughout culture conditions and S-ODN treatments. In conclusion, the present study provides a direct evidence for the potential antiviral activity of antisense oligonucleotides on native genomic replication of HCV genotype 4, the most common type in Egypt.

# ***Dedication***

***I dedicate this work  
To my family:***

***My Kind Mom  
My Great Dad  
My Dearest Husband  
My sweetheart Karim***

***I have to thank God for choosing you to be my family  
I can not imagine how was my life without you  
Thank you for your presence in my life  
Thank you for supporting me  
With kindness, patience,.....and love***

***Yours  
Noha Gamal El Din Samy.***

*To My Dear Husband  
Indeed I have to thank God  
for choosing you to be my husband*

*I am appreciate & grateful for  
everything you do it for me*

*Thanks a lot  
For your  
Encouragement  
Assistance  
Patience  
Care  
And  
Love*

*To My little angle*

*Karím*

*I hope one day,  
You read this thesis  
& be proud that  
I'm your mother*

*Thanks God  
For the Gorgeous  
& Precious Gift  
My sweetheart*

*Karím*

*Who fill my life with  
Happiness  
Smiles  
Blesses*

# ***ACKNOWLEDGMENT***

I would like to express my thanks and gratitude to **Professor Dr. Zeinab Z. El Dardiri** Professor Biochemistry, Faculty of Science, Ain Shams University (**God Rest her Soul**).

My deepest heartfelt gratefulness is to **Professor Dr. Mostafa K. El Awady**, Professor of Molecular Genetics, Biomedical Technology Department, National Research Center, for suggesting the point of this thesis, building up the hypothesis related to the results. Also, I thank him for his kind supervision continuous support and valuable guidance in all of the theoretical and practical aspects of this work. I am very lucky to have great opportunity to be one of his students.

I would like also to express my deepest appreciation, gratefulness & sincere thanks to **Dr. Gamila M. Labib Shanab**, Assistant Prof. of Biochemistry, Faculty of Science, Ain Shams University for her kind supervision, help, encouragement, guidance and advice. I am very glad to be one of her students.

I would like also to thank **Dr. Wael EL Garf**, Researcher, Biomedical Technology Department, National Research Center, for his help & support.

My endless thank and appreciation to my dear colleague **Jasmine El Abd** Research assistant, Biomedical Technology Department, National Research Center for her valuable effort, encouragement, kind and love. I ask God to reward & bless her. I wish to be friend for ever.

Finally, I would like to express my deep thanks to everybody in Biomedical Technology Department, who helped me directly or indirectly.



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### ***Arabic Summary***

### ***Arabic Abstract***

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## **LIST of ABBREVIATIONS**

A	Adenine
ALT	Alanine Transferase
AML	Acute Myelogenous leukemia
Anti-HCV	HCV antibody
AS-ODN	Antisense Oligodeoxynucleotides
ATCC	American Type Culture Collection
bp	Base pair
bDNA	Branched Deoxy ribonucleic acid
C	Cytosine
CaCl <sub>2</sub>	Calcium Chloride
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CMV	Cytomegalovirus
CO <sub>2</sub>	Carbon Dioxide
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dd H <sub>2</sub> O	Double distilled water
DEPC	Diethylpyrocarbonate
dGTP	Deoxyguanosine triphosphate
DMEM	Dulbecco's Modified Eagle's Medium
DTT	Dithiothreitol
dTTP	Deoxythymidine triphosphate
dUTP	Deoxyuracil triphosphate
DNA	Deoxy ribonucleic acid
E1	Envelope protein 1
E2	Envelope protein 2
EASL	European Association for the Study of the Liver
EDTA	Ethylene diamine tetra acetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay

ETOH	Ethanol
ETR	End of therapy response
FCS	Fetal Calf Serum
FDA	Food and Drug Administration
G	Guanine
g	gram
GAPDH	Glyceraldehyde-3- Phosphate Dehydrogenase
h	hours
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCl	Hydrochloric acid
HCMV	Human cytomegalovirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HVR1	Hypervariable region 1
IDU	Injection drug users
IFN	Interferon
IL	Interleukin
IMPDH	Inosine monophosphate dehydrogenase
IPTG	Isopropyl- $\beta$ -thiogalactopyranoside
IRES	Internal ribosome entry site
KCl	Potassium Chloride
KDa	Kilo Dalton
KOAc	Potassium Acetate
KPO <sub>4</sub>	PotassiumPhosphate
LDL	Low density lipoprotein
M	Molar
M2	Viral membrane matrix protein,
mg	Milligram
MgCl <sub>2</sub>	Magnesium Chloride
$\mu$ g	Microgram