"Studying the Role of Programmed Cell Death-1 and Interleukin-28B Genetic Polymorphism in Treatment Outcome of HCV"

A thesis submitted for the partial fulfillment of Philosophy Degree (Ph.D) in Pharmaceutical Sciences (Biochemistry)

Submitted By

Hanan Hosny Mahmoud Rizk

Assistant lecturer of Biochemistry, Faculty of Pharmacy, Ain Shams University,
Master Degree in Biochemistry, Ain Shams University, 2012
Bachelor of Pharmaceutical Sciences, Ain Shams University, 2007

Under Supervision of

Prof.Dr. Hala Osman EL-Mesallamy

Professor of Biochemistry
Head of Biochemistry Department
Faculty of pharmacy
Ain Shams university

Prof.Dr. Nadia Lotfy Al-Ansari

Professor of Gastroenterology and Hepatology
Gastroenterology and Hepatology Department
Faculty of medicine
Ain Shams university

Dr. Nadia Hamdy El-Hefny

Associate Professor of Biochemistry
Biochemistry Department
Faculty of Pharmacy
Ain Shams University

Biochemistry Department
Faculty of Pharmacy
Ain Shams University
2016

" دراسة دور تعدد الأشكال الجينية لجين موت الخلية المبرمج-١ والإنترليوكن-٢٨ب فى مخرجات علاج التهاب الكبد فيروس سى "

رسالة مقدمة كمتطلب جزئي للحصول على درجة دكتوراة الفلسفة في العلوم الصيدلية

(تخصص كيمياء حيوية)

مقدمة من

حنان حسنى محمود رزق

المدرس المساعد بقسم الكيمياء الحيوية- كلية الصيدلة- جامعة عين شمس
ماجستير كيمياء حيوية-جامعة عين شمس-٢٠١٢
بكالوريوس العلوم الصيدلية-جامعة عين شمس-٢٠٠٧

تحت إشراف

أ.د/ هالة عثمان المسلمى

أستاذ الكيمياء الحيوية
رئيس مجلس قسم الكيمياء الحيوية
كلية الصيدلة
جامعة عين شمس

أ.د/نادية لطفي الأنصاري

أستاذ الجهاز الهضمى والكبد
قسم الجهاز الهضمى والكبد
كلية الطب
جامعة عين شمس

أ.م.د/نادية حمدى الحفنى

أستاذ مساعد الكيمياء الحيوية
قسم الكيمياء الحيوية
كلية الصيدلة
جامعة عين شمس

قسم الكيمياء الحيوية
كلية الصيدلة
جامعة عين شمس

٢٠١٦

Acknowledgement

I truly thank **Allah** for His kind help, guidance and enlightening my way to accomplish this work. May it be a step towards gaining His mercy and blessings and may **Allah** accept from us the good deeds, and forgive us in shortcomings.

My deepest thanks and gratitude goes to my **Prof. Dr. Hala Osman El-Mesallamy, Professor and Head of Biochemistry Department, Faculty of Pharmacy, Ain-Shams University,** for her dedicated supervision, precious time, great efforts, faithful advice, continuous unlimited support and encouragement, and kind gestures in every moment throughout all this work. May it be added to her good deeds.

Blessed I am to work under the supervision of **Prof. Dr. Nadia Al-Ansari, Professor of Gastroenterology and Hepatology, Gastroenterology and Hepatology department, Faculty of Medicine, Ain-Shams University.** Indeed I learned a lot from her as a hepatology field pioneer. I am quite grateful and truly thank her for giving me the chance and help in collecting the samples from her clinic and **Yassin Abdul Ghaffar Center**, as well as her kind help, giving me from her precious time.

I would also like to express my gratefulness and appreciation for **Dr. Nadia Hamdy El-Hefny, Assistant Professor of Biochemistry, Faculty of Pharmacy, Ain-Shams University,** for her continuous, kind, priceless help, quick feedbacks and guidance in every step throughout this work. I wish her all the success.

I am truly thankful for **Dr. Aisha Yassin, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, the members of Yassin Abdul Ghaffar center and Dr Nadia Al Ansari's lab and clinic members** for their kind cooperation, facilitating the work a lot and were really a supporting helpful and very cooperative family. May Allah bless them all.

Never to be forgotten or repaid back; the priceless help, patience, support and understanding of my **family** during the tedious periods of this work.

Abbreviation	Definition
AFP	Alpha-fetoprotein
BMI	Body mass index
CD	Cluster of differentiation
CTL	Cytotoxic T lymphocytes
CTLA-4	Cytotoxic T lymphocyte antigen-4
DAAs	Direct acting antivirals
DCs	Dendritic cells
EIA	Enzyme immunometric assay
ELISA	Enzyme-linked immunosorbant assay
EVR	Early virologic response
GWAS	Genome wide association studies
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HOMA	Homeostatic model assessment
HWE	Hardy–Weinberg equilibrium
IL	Interleukin
IR	Insulin resistance
ISGs	Interferon stimulated genes
ITIM	Immunoreceptor tyrosine-based inhibitory motif
ITSM	Immunoreceptor tyrosine-based switch motif
JAK-STAT	Janus kinase-signal transducer and activator of transcription
MGB	Minor groove binder
NR	Non responders
NS	Non structural
OR	Odds ratio
PCR	Polymerase chain reaction
PD-1	Programmed cell death-1

PD-L	Programmed cell death ligand
PEG-IFN-α	Pegylated interferon alpha
RBV	Ribavirin
ROC	Receiver operating characteristic
RUNX1	Runt-related transcription factor 1
RVR	Rapid virologic response
SD	Standard deviation
SNPs	Single nucleotide polymorphisms
SVR	Sustained virologic response
TCR	T cell receptor
Treg	T Regulatory
Th	T Helper

List of Tables

List of Figures

1. Introduction and Aim of the Work

Hepatitis C virus (HCV) is a highly persistent human pathogen that infects the liver of 130-150 million patients worldwide, with yearly 350,000 deaths due to all HCV-related causes ***(WHO, 2015).*** The largest viremic populations are in Egypt, with prevalence of almost 15%, reaching staggering prevalence rates of 20% in highly endemic areas, including urban centers and the Nile Delta ***(Miller and Abu-Raddad, 2010; Bruggmann et al., 2014).*** Moreover, around 20% of worldwide HCV cases are of genotype 4 that constitutes 90% of HCV infections in Egypt ***(CDA, 2014).***

Although successful use of direct-acting antivirals (DAAs) was recently reported in Western countries, the high cost, limited trials and possible resistance on long terms represent a challenge to worldwide implementation ***(Feeney and Chung, 2014).*** Moreover, in Egypt new approved regimens of DAAs are combined with the standard conventional treatment of pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) to minimize the development of viral breakthroughs and relapse ***(Barth, 2015).*** Thus so far, the combined treatment with PEG-IFN α+RBV remains a cornerstone in treatment for patients with chronic HCV in Egypt ***(EASL, 2015).*** Nevertheless, fewer than half of patients are able to achieve sustained virologic response (SVR) ***(Hadziyannis et al., 2004),*** defined as an undetectable HCV RNA level 6 months after treatment discontinuation ***(Ghany et al., 2009).***

Treatment failure is likely to occur due to inherent viral and host factors such as the presence of certain single nucleotide polymorphisms (SNPs) and inappropriate drug regimens ***(Strahotin and Babich, 2012)***. Genome-wide association studies (GWAS) identified SNPs in proximity to the promoter for the *interleukin-28B* (*IL28B)* gene on chromosome 19, a human gene of host system innate antiviral defense, to be the most important predictor of achieving SVR ***(Rauch et al., 2010; De Nicola et al., 2012)***. However, *IL28B* gene alone is not a perfect predictor for informing treatment decisions ***(Ogawa et al., 2012)***. Moreover, studies that evaluated its role in conjunction with other markers on genotype 4 especially in Egypt are still few ***(Youssef et al., 2014)***.

Emerging studies show that T cell exhaustion occurring in viral infections correlates well with increased expression levels of co-inhibitory receptors including, programmed cell death-1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) ***(Intlekofer and Thompson, 2013; Wang et al., 2014)***. Some genetic SNPs were found to cause changes in the expression of these molecules and thus modulating immune response ***(Wang et al., 2008; Kristjansdottir et al., 2010)***. Previous studies have linked SNPs in *CTLA-4* gene to HCV severity and treatment outcome in HCV genotype 1 ***(Yee et al., 2003; Danilovic et al., 2012)***. Additionally, *PD-1.3/*A allele was denoted to be associated with SVR and to increase the predictive value of *IL28B* C/C genotype in HCV genotypes 1 and 3 ***(Vidal-Castineira et al., 2012)***.

Based on the aforementioned considerations, this study aimed to:

1- Investigate the association of pretreatment clinical factors with the efficacy of PEG-IFN-α+RBV treatment in Egyptian patients with chronic HCV genotype 4 infection.

2- Explore *IL28B* (rs12979860), *PD-1.3* (rs11568821) *and CTLA-4* 49A/G (rs231775) genetic SNPs, their correlation with SVR and clinical value in Egyptian patients with chronic HCV genotype 4.

3- Build a model to predict SVR probability.

2. Literature Review

2.1 Epidemiology and Natural History of HCV Infection

Hepatitis C virus is a small (55-65 nm) enveloped ssRNA virus of the flaviridae family, a globally prevalent pathogen and a leading cause of morbidity and mortality. Hepatitis C virus has been given many names, ‘the silent epidemic', ‘the silent dragon' and ‘the disease of the new millennium" ***(Nadeem et al., 2010)***. Estimates of disease burden show an increase in seroprevalence over the last 15 years to 3%, equating to >185 million infections worldwide ***(Mohd Hanafiah et al., 2013),*** with 3–4 million yearly new infections ***(WHO, 2015).***

On the other hand, as shown in figure 1, Egypt is confronted with an HCV disease burden of historical proportions, where Egypt has the highest HCV prevalence in the world with a prevalence 10-folds greater than in the United States and Europe and total estimated number of around 12 million infected Egyptians ***(Mohamoud et al., 2013; Mohd Hanafiah et al., 2013).*** These numbers are probably an underestimate, since many HCV-infected individuals have not been tested and thus remain undiagnosed ***(Abdel-Hakeem and Shoukry, 2014).***

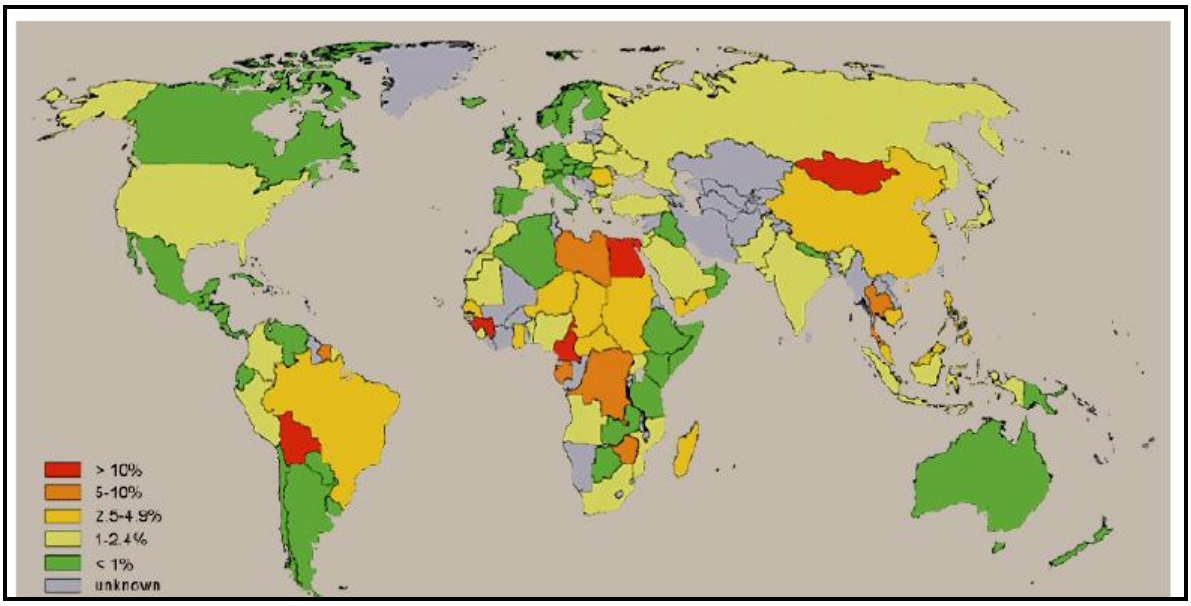

Figure (1): Worldwide prevalence of HCV infection *(Mohd Hanafiah et al., 2013).* *[Percentages represent prevalence of HCV antibodies]*.

Not only that, but as opposed to other countries where HCV dynamics is focused in specific high risk groups, such as intravenous drug users, HCV transmission in Egypt has reached diverse population groups including those not conventionally identified to be at risk of infection ***(Guerra et al., 2012).*** It has been postulated that the epidemic was caused by extensive iatrogenic transmission during the era of parenteral antischistosomal therapy mass treatment campaigns ***(Frank et al., 2000).*** Being one of the top five leading causes of death of Egyptians, HCV infection and its complications are among the leading public health challenges in Egypt ***(Lavanchy, 2011).***

There are six HCV genotypes that differ from each other by 30-35% of nucleotide sequence and which can be split into multiple subtypes ***(Simmonds et al., 2005).*** A recent study, encompassing data of 90% of the world population, showed that HCV genotype 1 accounted for almost half of

HCV cases in the world, with more than one-third of cases reported in East Asia. Genotype 3 was the second most common, with about 75% of cases reported in South Asia. Third was genotype 2, which is reported primarily in East Asia; while genotype 4 came in forth place, reported primarily in North Africa; followed by 6, reported primarily in East Asia. Genotype 5 accounted for less than 1% of cases worldwide, with the majority reported in Southern and Eastern sub-Saharan Africa as shown in figure 2 ***(Messina et al., 2015)*.**

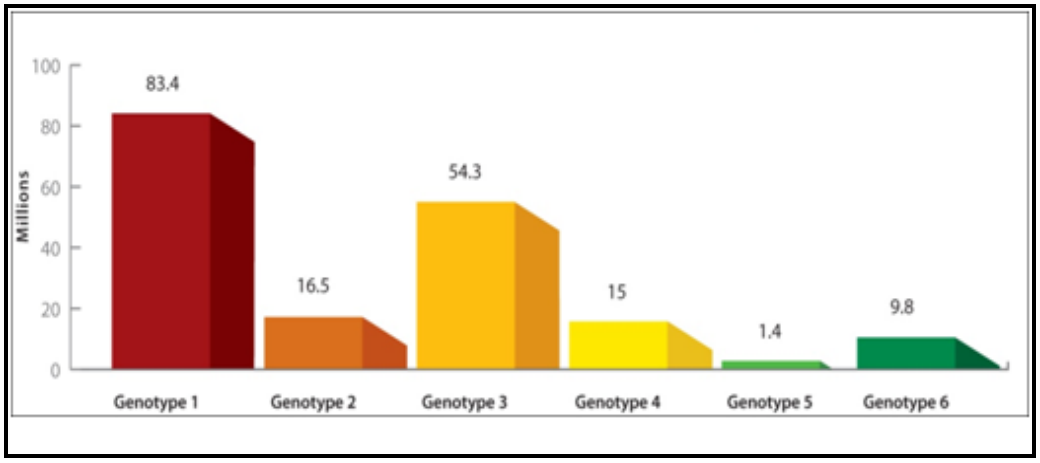

Figure (2): Prevalence of HCV genotypes *(Messina et al., 2015).*

Sixty-six percent of genotype 1 cases were reported in countries classified by WHO as high-income, whereas genotypes 4 and 5 were more common in countries classified as low-income ***(Messina et al., 2015)*.**

According to the ***CDA (2014***) genotype 4 is primarily confined in Egypt and constitutes 90% of HCV infections as shown in figure 3. Most of the cases reported were acquired via contaminated needles in the anti-schistosomiasis program or with contaminated blood transfusion.