"Studying the Role of some Interferon-Stimulated and Vitamin D Related Genes Polymorphism in Treatment Outcome of HCV"

A Thesis Submitted for the Partial Fulfillment of Doctor of Philosophy Degree in Pharmaceutical Sciences (Biochemistry)

by

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Abbreviation	Definition
ADAR	Adenosine deaminases acting on ribonucleic acid
AFP	Alpha-fetoprotein
AUROC	Area under the receiving operating curve
BMI	Body mass index
CD	Cluster of differentiation
CHC	Chronic hepatitis C
CYP2R1	Cytochrome P-450 family2, subfamilyR, polypeptide1
DAAs	Direct acting antivirals
DBP	Vitamin D-binding protein
dsRNA	Double-stranded ribonucleaic acid
EASL	European association for the study of the liver
G	Genotype
GWAS	Genome-wide association studies
HALT-C	Hepatitis C antiviral long-term treatment against cirrhosis
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HOMA-IR	Homeostasis model assessment for insulin resistance
HWE	Hardy–Weinberg equilibrium
IFNAR	Interferon A receptor
IFN-α	Interferon-alpha
IL	Interleukin
IMPDH	Inosine-monophosphate-dehydrogenase inhibition
IRF	Interferon regulatory factor
IRS1	Insulin receptor substrate 1
ISGs	Interferon stimulated genes
IκB	Inhibitor of kappa beta
JAK-STAT	Janus activated kinase- signal transducer and activator of transcription
MGB	Minor groove binder
NK	Natural killer
NF- κB	Nuclear factor-kappa B cell
NR	Non-response
NS	Non-structural protein
OAS	Oligoadenylate synthetase
OASL	Oligoadenylate synthetases-like
OR	Odds ratio
PAMP	Pathogen-associated molecular patterns
Peg-IFN	Pegylated interferon-alpha

PKR	Protein kinase ribonucleic acid-activated
PRRs	Pattern-recognition receptors
RBV	Ribavirin
RIG-1	Retinoic acid-inducible gene 1
RMP	Ribavarin monophosphate
RNase L	Ribonuclease latent
RTP	Ribavarin triphosphate
RT-PCR	Real time reverse transcription polymerase chain reaction
RVR	Rapid viral response
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
SVR	Sustained viral response
Th	Thymocytes-helper cell
TLC	Total leukocyte count
USP 18	Ubiquitin-specific peptidase 18
URT	Untranslated region
VDR	Vitamin D receptor

Introduction and Aim of the Work

Hepatitis C virus (HCV) is a hepatotropic non-cytopathic positive strand ribonucleic acid (RNA) virus, which represents a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). It affects 3% of the world's population where about 170 million are chronic carriers ***(Gower et al., 2014)***. The largest viremic populations are found in Egypt where it is rated as the fourth country contributing to more than half of total global infections ***(Bruggmann et al., 2014)***.

Pegylated interferon-alpha (Peg-IFN) plus ribavirin (RBV) constitutes the standard backbone treatment for chronic hepatitis C (CHC). Nevertheless fewer than half of patients are able to achieve sustained viral response (SVR) while the rest of patients don't show response or experience severe complications ***(Ghany et al., 2009)***.

By the introduction of the novel direct acting antivirals (DAAs), CHC treatment modalities dramatically evolve increasing rates of SVR, treatment efficacy and tolerability especially in patients with advanced liver disease (fibrosis METAVIR score F3 or F4). However, the burden of chronic infection set a questionable issues about its availability and its cost-effectiveness ratio in treating all CHC patients ***(Camma et al., 2012)***.

Recently, according to the latest guidelines of the European Association for the Study of the Liver (EASL) six treatment options are available for patients infected with HCV genotype (G) 4, including two interferon-containing regimens which are sofosbuvir and simeprevir and four interferon-free regimens which are combinations of sofosbuvir and simeprevir or sofosbuvir and ledipasvir or ombitasvir, paritaprevir and ritonavir or sofosbuvir and daily daclatasvir ***(EASL, 2015)***. But ***EASL***

(2015) declared that the combination of PegIFN and RBV remains acceptable in settings where non of these options is available.

Thus, despite progress in DAAs there is still room for optimizing the efficacy of the less costly standard basic therapy in naïve CHC patients. This aim could be obtained by further refining and identification of the treatment factors associated with maximal outcome benefits from it ***(Falleti et al., 2013)***.

Several predictors of successful treatment response of CHC are identified. Some of these depend on the virus itself others are host-related, either genetic (interleukin 28B (IL28B) rs12979860 gene polymorphisms, gender and race) or acquired (insulin resistance, obesity, liver steatosis and liver fibrosis stage) ***(Asselah et al., 2010; Maekawa et al., 2012; Garcia-Martin et al., 2013)***.

Vitamin D appears to possess an important immune-modulator effect beside its classical action on calcium metabolism ***(White, 2012)***. Although its deficiency is very common in CHC patients ***(Miroliaee et al., 2010)*** yet, conflicting results were found by relating these deficiencies with the success of Peg-IFN and RBV therapy ***(Bitetto et al., 2011; Kitson et al., 2013)***. Vitamin D undergoes two activation processes before its interaction with vitamin D receptor (VDR). The first activation is performed in the liver by cytochrome P-450 family 2, subfamily R, polypeptide 1 (CYP2R1) and produces 25-hydroxylated form of vitamin D ***(Holick, 2011)***. This step produces the main circulating vitamin D form in serum. By means of CYP27B1, vitamin D is subjected to its second hydroxylation which leads to the production of 1,25(OH) active vitamin D form which interacts with its specific transmembrane receptor VDR to exert its physiological functions ***(Rosen et al., 2012)***.

Cytochrome P-450 family 2, subfamily R, polypeptide 1 and VDR genes having different polymorphic forms, CYP2R1 and VDR are recently suggested to influence the efficacy of antiviral therapy. On the other hand functional single nucleotide polymorphism (SNP) of CYP27B1 gene fails to predict antiviral response in CHC patients mainly G1 ***(Falleti et al., 2013; Garcia-Martin et al., 2013)***.

A more recent study found that VDR crosstalk the Janus activated kinase- signal transducer and activator of transcription proteins **(**JAK–STAT) pathway through altered expression of interferon stimulated genes (ISGs) which results in calcitriol-mediated increase of hepatocellular response to interferon-alpha (IFN-α) ***(Lange et al., 2014)***. Where, signaling the expression of ISGs by the administration of exogenous interferon provides antiviral action against HCV through interferon receptors and through Jak–STAT pathway ***(Feld and Hoofnagle, 2005)***.

Of these ISGs functional SNPs at oligoadenylate synthetases-like (OASL) gene and adenosine deaminases acting on RNA (ADAR) gene were found to affect the response to antiviral therapy of HCV G1 ***(Su et al., 2008; Welzel et al., 2009)***.

Knowing that different HCV genotypes respond differently to interferon antiviral therapy ***(Holmes et al., 2015)*** there are 6 main identified HCV genotypes of which more than 94% of the Egyptian patients are infected by G4 ***(Gower et al., 2014)***. Accordingly, this present study aimed to investigate for the first time the influence of SNPs in CYP2R1, VDR, OASL, ADAR and IL28B genes as well as serum 25-OH vitamin D levels, serum levels of alpha-fetoprotein (AFP) and insulin resistance on treatment outcomes of Egyptian CHC G4 patients treated with Peg-IFN and RBV.

1. Literature Review

Hepatitis C virus is a major cause of chronic hepatitis and the leading cause of end stage liver disease including liver cirrhosis and HCC ***(Georgel et al., 2010)***. It has a positive sense single-stranded RNA genome. The genome consists of a single open reading frame that is 9600 nucleotide bases long ***(Kato, 2000)***.

Virological studies have identified six HCV genotypes, and a large number of subtypes ***(Simmonds et al., 2005)***. Genotype 1 (subtypes 1a and 1b) is the most prevalent genotype worldwide, with a higher prevalence of 1b in Europe and 1a in the United States. Genotype 2 is found in clusters in the Mediterranean region, G3 is highly prevalent in European intravenous drug users in whom the prevalence of G4 is also increasing. Egypt has the highest prevalence of G4. Finally, G5 and G6 are less frequent ***(Esteban et al., 2008, Ashfaq et al., 2011)***. Genotypes differ by 30–35% of the nucleotide sites over the complete genome ***(Nakano et al., 2012)***. Genotype is clinically important in determining potential response to IFN-based therapy and the required duration of such therapy ***(Rose et al., 2013)***.

According to the clinical practice guideline of the ***EASL (2012)*** the principal routes of HCV transmission have changed from blood transfusions and unsafe injections or surgical procedures to intravenous and tattooing or acupuncture with unsafe materials in the last 20 years.

2.1. Chronic Hepatitis C:

Chronic infection with HCV is a leading indicator for liver disease. Only 20%–30% of hepatitis C infected persons are expected to resolve following the acute phase without any therapeutic intervention while the remaining 75%–85% develop CHC within 6 months of infection as shown in figure (1) ***(Chen and Morgan, 2006)***.

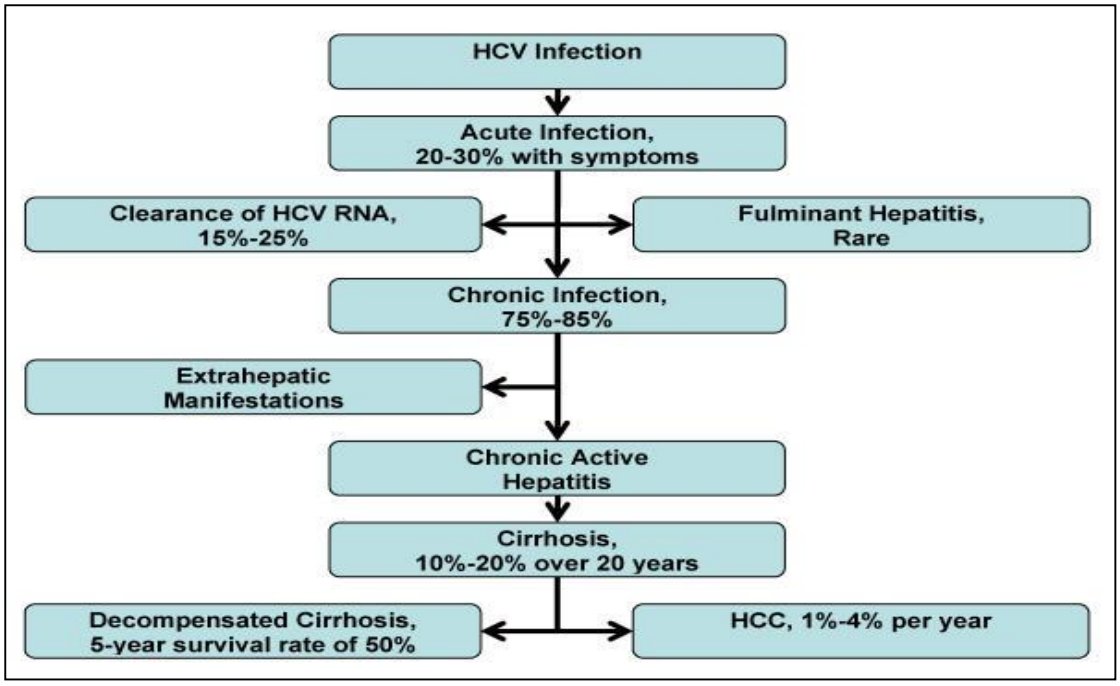

Figure (1): Natural history of HCV *(Chen and Morgan, 2006)*.

Chronic HCV infection is associated with variable degrees of hepatic inflammation and progression of fibrosis, whatever the HCV genotype or viral load. Depending on the presence of co-factors, between 10% and 40% of patients with chronic HCV infection will develop cirrhosis ***(Afdhal, 2004)***. Death related to the complications of cirrhosis occurs at an incidence of approximately 4% per year, whereas HCC occurs in this population at an estimated incidence of 1–5% per year ***(Thompson Coon et al., 2007)***.

Development of chronicity correlates with the evolution of HCV genomes having varied sequences that have presumably escaped immune surveillance. Chronically infected people can remain asymptomatic for several years, or even for life, and many infected patients do not know until they develop liver disease, cirrhosis, and HCC ***(Ghany et al., 2009)***. That's why CHC has been recognized as a silent epidemic ***(Nadeem et al., 2010)***.

2.1.1 Epidemiology :

Geographic distribution of HCV is not uniform as shown in figure (2) ***(Wedemeyer et al., 2015)***. Three to four million new infections occur each year and about 170 million people are chronically infected due to liver diseases including cirrhosis and liver cancer ***(WHO, 2015)***. Where, 500 thousand deaths occur each year due to all HCV-related disorders ***(Bruggmann et al., 2014)***.

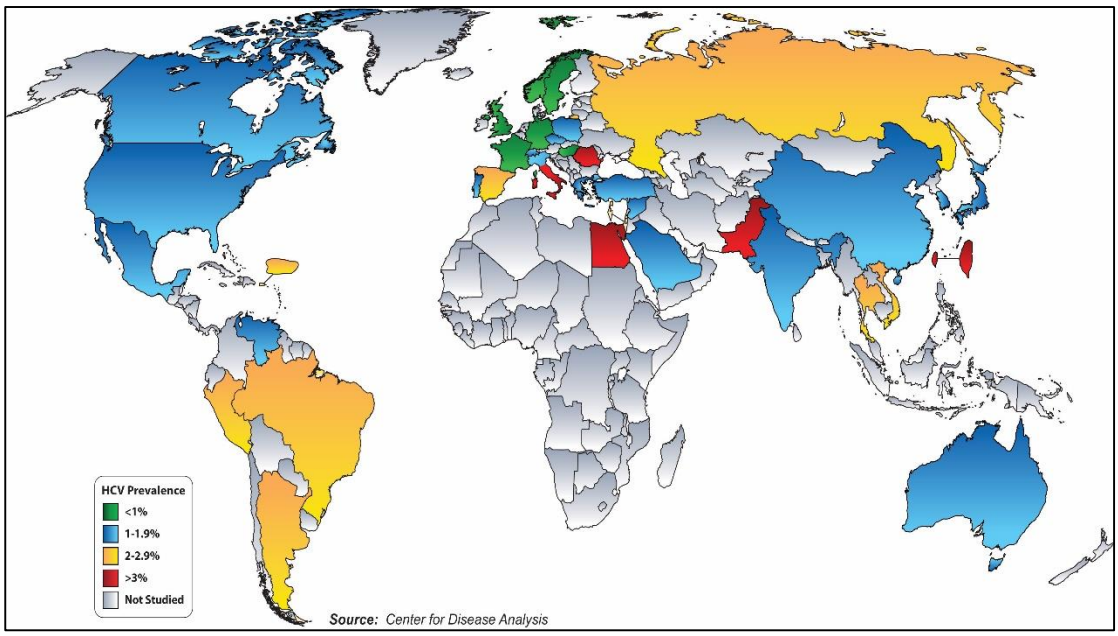

Figure (2): Global estimated prevalence of HCV *(Wedemeyer et al., 2015)*.

Egypt has by far the highest national-level HCV prevalence in the world, with more than 14% of the Egyptian adult population having been exposed to the virus ***(Mohamoud et al., 2013)***. About 21% of the Egyptian