INTRODUCTION

There are approximately 71 million chronically HCV infected individuals worldwide, many of whom are unaware of their infection, with important variations according to the geographical area ***(EASL, 2018).***

HCV infection is a major cause of liver cirrhosis, hepatocellular carcinoma (HCC) and end-stage liver disease. For the past two decades chronic hepatitis C was treated with interferon alpha (IFNa) and ribavirin (RBV). IFNa/ RBV combination therapy was associated with frequent and sometimes severe side effects. Tolerability was a particular problem in patients with advanced liver fibrosis and IFNa/RBV was even contraindicated in decompensated cirrhosis ***(Manns et al., 2006).*** Thus, treatment of HCV infection in patients with the most urgent clinical need was not possible before the approval of IFNa-free treatment with direct acting antiviral (DAA) drugs against HCV ***(Deterding et al., 2015).***

In January 2014, sofosbuvir has been approved by the European Medical Agency (EMA) as the first pangenotypic once-daily NS5B polymerase inhibitor for the treatment of patients with chronic hepatitis C. Sofosbuvir plus ribavirin without interferon is recommended by international guidelines for patients with HCV genotype 2 (12 weeks) ***(Lawitz et al., 2013)*** and genotype 3 (24 weeks) ***(Zeuzem et al., 2014)*** and may also lead to high cure rates in HCV genotype 4 infection

when given for 24 weeks ***(Ruane et al., 2015).*** However, there is no information if this combination is also effective in more advanced stages of liver cirrhosis. In addition, sofosbuvir and ribavirin has not been studied in larger cohorts of patients infected with HCV genotype 1 ***(Deterding et al., 2015).***

The second generation protease inhibitor simeprevir and the first HCV-NS5A inhibitor (daclatasvir) were approved by EMA in May and in August 2014 respectively. Simeprevir and sofosbuvir can be used in combination for 12 weeks in HCV genotype 1 patients with an urgent need of antiviral therapy based on a phase-2 proof-of-concept trial ***(Lawitz et al., 2014).*** Subsequent 'real-world' experiences confirmed the high efficacy of this specific combination with cure rates of greater than 80% ***(Jensen et al., 2014).***

Daclatasvir was evaluated in combination with sofosbuvir for previously treated or untreated patients in a phase 2 trial where almost all patients were cured and only a single genotype 3 infected patients experienced a documented virological treatment failure ***(Sulkowski et al., 2014).*** However, liver cirrhosis was an exclusion criteria in this study. A subsequent trial investigating 12 weeks of sofosbuvir plus daclatasvir in HCV genotype 3 infection showed lower responses of only 58–69% in patients with liver cirrhosis as compared to 96% in non cirrhotic individuals. Neither for simeprevir nor for daclatasvir full study reports are available for patients with Child-Pugh B or C cirrhosis ***(Nelson et al., 2015).***

Successful interferon-based antiviral therapy of hepatitis C patients with advanced fibrosis and compensated liver cirrhosis is associated with decreased incidence of HCC, hepatic decompensation and liver-related mortality and may even lead to a survival comparable with that of the general population ***(Veldt et al., 2012).*** However, it is unknown to what extent liver function may improve with interferon-free therapies in advanced liver cirrhosis when peg-IFNa is contraindicated ***(Deterding, et al., 2015).***

One may also question if there is a 'point-of no- return' when HCV treatment is no longer useful in decompensated cirrhosis. Successful antiviral treatment of decompensated hepatitis B with HBV polymerase inhibitors has been shown to be associated with improvement of liver function and may even lead to de-listing of patients on the transplant waiting list ***(Jang et al., 2015).*** However, it remains to be shown if suppression of viral replication would lead to similar clinical improvements in hepatitis C cirrhosis. Moreover, many IFN-free treatment regimens still include ribavirin which may cause side effects particularly in patients with decompensated cirrhosis ***(Deterding et al., 2015).***

AIM OF THE STUDY

The aim of the present study is to evaluate the effect of direct acting antivirals (DAAs) on the synthetic functions of the liver in the treatment-naïve Egyptian patients with chronic hepatitis C genotype 4.

Chapter 1
CHRONIC HEPATITIS C INFECTION

Introduction:

Chronic hepatitis C virus (HCV) infection is a public health problem, with about 71 million people infected worldwide. It is a leading cause of liver related morbidity and mortality through its predisposition to liver fibrosis, cirrhosis, and liver cancer ***(Renau et al., 2018).***

HCV is a positive sense, single-stranded RNA virus of the Flaviviridae family, which also includes many arthropod-borne human pathogens of the Flavivirus genus such as yellow fever virus, West Nile virus and dengue virus ***(Quan et al., 2013).***

HCV isolates have been grouped into seven genotypes and a number of subtypes with distinct geographic distributions and sensitivity to interferon-based treatment for a long time, the lack of a cell culture system was a major obstacle to study the HCV life cycle ***(Zein et al., 2000).*** However, selectable replicon systems and retrovirus-based pseudotyped particles have been major tools to understand HCV genomic replication and virus entry, respectively. Finally, since 2005, the full viral life cycle can be investigated with the help of complete viral replication systems ***(Jean and Cosset, 2014).***

It is transmitted parenterally. Transmission via the transfusion of whole blood and blood products has been minimized through the use of serological and molecular testing procedures. The risk of transmission of HCV now arises mainly from needle sharing among intravenous drug abusers, homosexual contacts, and, in countries with poor adherence to hygiene guidelines and iatrogenic transmission ***(Zeuzem et al., 2017).***

Acute hepatitis C takes a chronic course in 50–70% of cases. Hepatic fibrosis progresses is dependent on the age of the patient at the time of infection. On average, 20–30% of patients develop cirrhosis within 30 years. The progression of fibrosis is modulated by cofactors such as the amount of alcohol consumed or viral coinfections (e.g., with HIV). Patients with HCV-associated cirrhosis have a 3–6% incidence of hepatocellular carcinoma ***(Zeuzem et al., 2017).***

In addition to hepatic diseases, HCV infection has also been found to be involved in a variety of extra-hepatic diseases, affecting the kidneys, skin, salivary glands, eyes, thyroid, joints, nervous system, and immune system. Extra-hepatic diseases are reported in up to three quarter of patients and produce an important burden for society and health care systems ***(Renau et al., 2018).***

For many years, treatment for chronic HCV has been inadequate with success rates of treatment estimated at around 50%, depending on genotype. The standard of care until 2011

was a combination of pegylated interferon-alpha (PEG-IFN), administered subcutaneously and ribavirin (RBV) taken orally. This combination could lead to a sustained virological response (SVR) and, based on long-term follow up results, However, such treatment is associated with significant adverse events and furthermore, poorly tolerated and less efficacious in subjects with advanced disease who are at most need ***(Fried et al., 2002; Daniel et al., 2015).***

The introduction of direct acting antiviral drugs (DAAs), with two protease inhibitor drugs licensed in 2011, has improved treatment responses rates and heralded a new era of HCV treatment ***(Jacobson et al., 2011).*** A pipeline of new DAAs are in various stages of pre-clinical and clinical development creating great optimism for the future of managing chronic HCV infection with simple, short, interferon-free, all oral regimens ***(Asselah et al., 2013).***

The primary goal of HCV therapy is to cure the infection, i.e. to achieve a sustained virological response (SVR) defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment completion ***(EASL, 2018).***

Epidemiology:

Natural history, prevalence and modes of tramnsmission

HCV infection has a propensity to cause chronic hepatitis which may lead to cirrhosis, decompensated cirrhosis and HCC. The onset and accumulation of hepatic fibrosis is clinically silent in the early stages of disease, and it therefore remains difficult to accurately identify progression of the disease to cirrhosis in patient ***(Daniel et al., 2015).***

Annual rates of progression of hepatic fibrosis from minimal disease to cirrhosis have been modelled and estimated ***(Daniel et al., 2015).***

The prevalence of biopsy proven cirrhosis after 20 years of infection has varied between 7% (in retrospective studies) to 18% (in clinical referred settings). The risk of cirrhosis is increased in individuals abusing alcohol, in those who acquire the disease at an older age, by concomitant obesity, in men, and in immunosuppressed HIV positive patients or in recurrent HCV following liver transplantation ***(Thein et al., 2008).***

Patients with minimal fibrosis have a low risk of developing complications of liver disease over the ensuing two decades. Patients with bridging fibrosis or cirrhosis, conversely have a higher risk. It may be necessary to repeat liver biopsies in patients to determine progression. Alternatively and more

practically, non-invasive blood tests, fibroelastography and hepatic imaging can be used to identify patients with advanced fibrosis to gauge indications for immediate or deferred treatment ***(Castera et al., 2009; Daniel et al., 2015).***

Extrahepatic manifestations of HCV such as cryoglobulinaemia, or HCV-associated splenic lymphoma are also indications for antiviral therapy. Treatment will reduce infectivity and transmission in individuals using intravenous drugs ***(Daniel et al., 2015).***

Prevalence

There are approximately 71 million chronically infected individuals worldwide, many of whom are unaware of their infection, with important variations according to the geographical area ***(EASL, 2018).***

Each year, hepatitis C causes approximately 399, 000 deaths worldwide, mostly from cirrhosis and hepatocellular carcinoma (HCC) ***(Elakel et al., 2017).***

Prevalence of HCV infection is highly variable. It is found worldwide but with country prevalence ranging from less than 1% to greater than 10%. The highest prevalence has been reported in Africa and Middle-East, with a lower prevalence in the Americas, Australia, and Northern and Western Europe ***(Puchades et al., 2018).***

Hepatitis C virus (HCV) infection is a major health problem in Egypt as the nation bears the highest prevalence rate worldwide. In 2008, Egypt had the highest burden of hepatitis C virus (HCV) infection worldwide, where about 15% of the population were seropositive, 10% chronically infected, with 90% of patients infected with genotype 4. In 2015, the seroprevalence of HCV infection in Egypt has declined to 6.3% among the studied population with an overall estimated 30% decrease in HCV prevalence in Egypt between 2008 and 2015 ***(Elakel et al., 2017).***

Among Egyptian patients with chronic HCV, 35%-45% will develop some level of progressive liver disease, and without treatment approximately 5%-10% will develop cirrhosis (10%-20% lifetime risk) and 1%-3% develop hepatocellular carcinoma (HCC) ***(Elakel et al., 2017).***

Modes of HCV transmission

HCV has long been recognized as a parenterally transmitted cause of viral hepatitis ***(Klevens et al., 2012).*** Transmission via blood transfusion was a major route before the implementation of universal screening of blood in the developed world though this route remains a problem elsewhere ***(Zou et al., 2010).*** Where blood products and transplanted organs are now safe, intravenous drug use (IDU) has become the major route of HCV transmission ***(Cornberg et al., 2011).***

Epidemiological studies highlight transmission sources other than the sharing of contaminated needles, including the sharing of other drug paraphernalia such as foil and spoons**.** These findings are of vital importance in targeted prevention interventions ***(Thorpe et al., 2002; Daniel et al., 2015).***

Mother to child transmission

MTCT rates are estimated to be 2 to 8% in HCV monoinfected mothers but may be two to four times higher in those coinfected with HIV ***(Prasad et al., 2013).*** There are currently no data from randomized controlled trials to support recommending caesarian section in this setting ***(Daniel et al., 2015).*** The efficiency with which HCV is sexually transmitted has been controversial. However, in monogamous heterosexual couples where one partner has chronic HCV, the rate of transmission to a discordant partner is extremely low. Furthermore, HCV transmission is not associated with any particular sexual practice allowing for more unambiguous messages to be given to these couples ***(Terrault et al., 2013; Daniel et al., 2015).***

Genotypes

There are currently 7 established genotypes, although most work has focused on genotypes 1-6, which although showing global spread, have different geographical origins. Genotypes 1, 2, 4 and 5 are found as endemic infections in

Africa, whilst genotypes 3 and 6 have evolved in Asia ***(Simmonds et al., 2004).*** During the last century, a number of medical interventions such as schistosomiasis eradication campaigns in Egypt have amplified specific strains to epidemic proportions and subsequently many of these have spread internationally ***(Pybus et al., 2001).***

Virology

HCV Particle and genomic organization

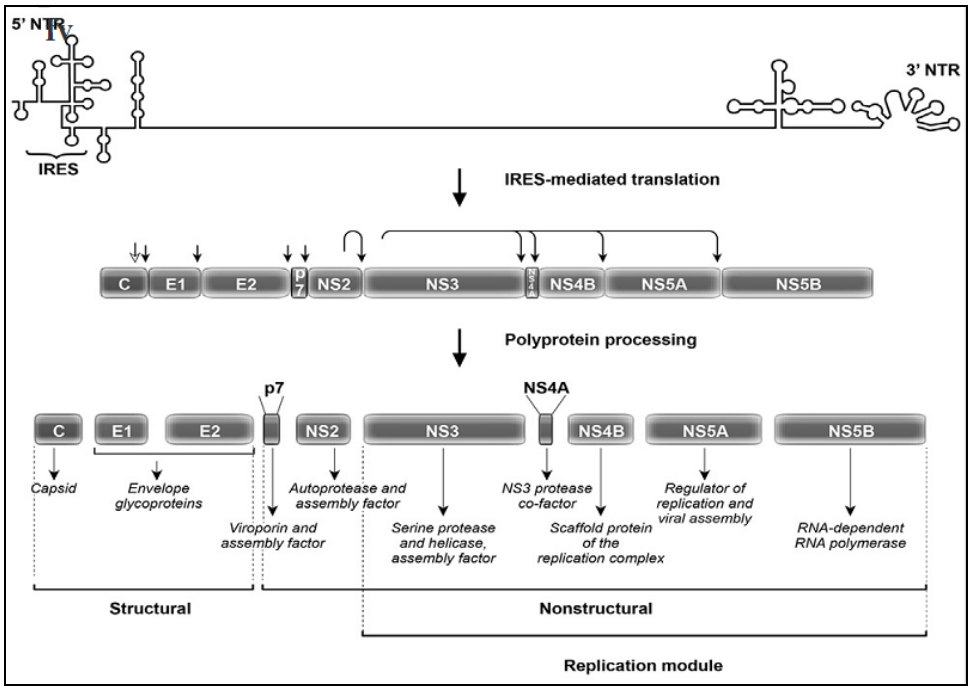

Fig. (1): HCV genome and proteins ***(Markus and Robert, 2014).***

HCV genome contains a single open reading frame flanked by 5′ and 3′ non-translated regions (NTRs). The 5′ NTR contains an internal ribosome entry site (IRES). After its

synthesis, HCV polyprotein is cleaved by viral and host encoded proteases. Cleavage in the N-terminal part of the polyprotein is mediated by cellular signal peptidases as indicated by individual vertical arrows. An additional cleavage removing the carboxy-terminal region of the core protein is mediated by cellular signal peptide peptidase, as indicated by an open arrow. The linked arrows indicate the cleavages by the viral proteases NS2 and NS3/4A. The functions of the individual proteins are indicated at the bottom of the figure ***(Dubuisson et al., 2014).***

Table (1): Structure and function of hepatitis C virus proteins ***(Moradpour and Penin, 2013)***

Pro-tein	Aa	MW (kDa)	Structure	Function
C	191	21	N-terminal basic, RNA-binding domain 1 (aa 1–117); hydrophobic, lipid droplet-binding domain 2 (aa 118 to ~177, harboring 2 amphipathic α-helices connected by a hydrophobic loop); C-terminal signal sequence. Maturation via signal peptide peptidase-mediated removal of the C-terminal signal sequence. Dimeric protein (stabilized by disulfide bond formation through Cys 128)	Nucleocapsid formation
E1	192	35	Highly glycosylated (up to 6 glycosylation sites), 4 potential disulfide bonds, C-terminal transmembrane domain	Envelope glycoprotein. Heterodimer formation with E2
E2	363	70	Highly glycosylated (up to 11 glycosylation sites), 9 potential disulfide bonds, C-terminal transmembrane domain. Hypervariable region 1 (aa 1–28). Binds directly to CD81 and scavenger receptor BI	Envelope glycoprotein. Heterodimer formation with E1
p7	67	7	Two transmembrane α-helices connected by a cytoplasmic loop. Forms oligomers (hexamer and heptamer)	Viroporin

Pro-tein	Aa	MW (kDa)	Structure	Function
NS2	217	23	N-terminal membrane domain with 3 predicted transmembrane passages. C-terminal catalytic domain (aa 94–217) forms a dimeric cysteine protease with two composite active sites	Membrane-associated dimeric cysteine protease
NS3	631	70	N-terminal serine protease domain (aa 1–180) with a chymotrypsin-like fold with two β-barrel subdomains. Includes a structural zinc binding site. NTPase/RNA helicase domain (aa 181–631) with 3 subdomains. Membrane association through helix α_0 and N-terminal transmembrane segment of NS4A	Serine protease and NTPase/RNA helicase activities. Forms a noncovalent complex with NS4A
NS4A	54	8	N-terminal transmembrane α-helix (aa 1-21); central part (aa 21–32) forms a β-sheet as part of the N-terminal β-barrel of NS3; C-terminal acidic portion (aa 40-54) interacts with other replicase components, including NS3 NTPase/RNA helicase domain	Cofactor for NS3
NS4B	261	27	Integral membrane protein comprising an N-terminal portion (aa 1 to ~69, including amphipathic α-helices AH1 and AH2, extending from aa 3-35 and aa 42-66, respectively), a central part harboring four predicted transmembrane passages (aa ~70 to ~190), and a C-terminal portion (aa ~191–261, including amiphipathic α-helices H1 and H2, extending from aa 201–213 and 229–253, respectively)	Induction of the membranous web. NTPase activity? RNA binding?
NS5A	447	56–58	N-terminal amphipathic α-helix as membrane anchor (aa 1–31); domain 1 (aa 36–213) includes a zinc binding site and forms either a 'claw-like' dimer with a basic groove or a side-by-side dimer; both natively unfolded domains 2 (aa 250–352) and 3 (aa 356–447) exhibit intrinsic α-helical propensity	Serine phosphoprotein; RNA binding; phosphorylation-dependent modulation of RNA replication (domains 1 and 2) and viral assembly (domain 3)
NS5B	591	68	Fingers, palm, and thumb subdomains. Interactions between fingers and thumb subdomains result in encircled active site. Membrane association mediated by C-terminal transmembrane α-helix (tail-anchored protein)	RNA-dependent RNA polymerase

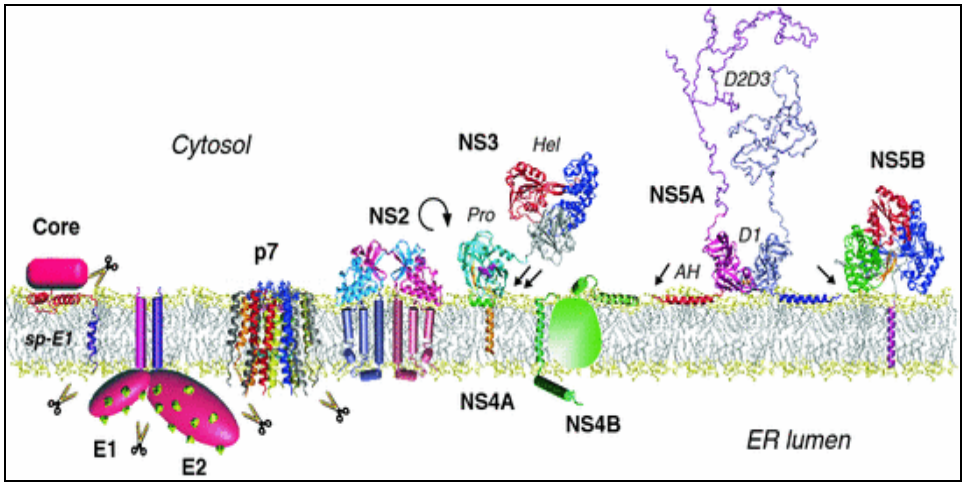

Fig. (2): Structure and membrane association of HCV proteins ***(Moradpour and Penin, 2013).***

HCV Particle:

HCV particles are 50–80 nm in diameter and contain the single-stranded RNA genome, core and the envelope glycoproteins, E1 and E2. The HCV genome interacts with the core protein to form the nucleocapsid that is surrounded by a lipid membrane, called the viral envelope, in which the envelope glycoproteins are anchored ***(Jean and Cosset, 2014).***

Importantly, due to virion association with lipoproteins, apolipoproteins such as apoE, apoB, apoA1, apoC1, apoC2, and apoC3 can also be found in association with HCV particles ***(Andre et al., 2002).***

Furthermore, a characterization of cell culture-produced particles indicates that their lipid composition resembles very-low density lipoproteins (VLDL) and low-density lipoproteins