CHAPTER ONE

HEPATITIS C VIRUS INFECTION

1.1 Introduction:

Hepatitis C virus (HCV) is a small-enveloped virus with one single-stranded positive-sense RNA molecule of approximately 9.6 kb. It is a member of the genus hepacivirus within the Flaviviridae family. Naturally occurring variants are classified into 6 major genotypes, each presenting with many subtypes **(*Simmonds, 2004, Simmonds et al., 2005 and Stapleton et al., 2011*).**

HCV is a life threatening human pathogen, not only because of its high prevalence and worldwide burden but also because of the potentially serious complications of persistent HCV infection ***(Bostan and Mahmood, 2010).***

1.2 Epidemiology:

Hepatitis C is a disease with a significant global impact. According to the World Health Organization (WHO) there are about 150 million people chronically infected with the HCV, corresponding to 2-2.5% of the world's total population. There are considerable regional differences; Egypt has the highest prevalence of HCV in the world, estimated nationally at 14.7% as shown in Figure1 ***(El-Zanaty and Way, 2009, Meffre et al., 2010, Hatzakis et al., 2011 and Mohamoud et al., 2013).*** In Africa and the western Pacific, the

prevalence is significantly higher than in North America and Europe **(*WHO, 2013*).**

HCV strains belonging to the major genotypes 1, 2, 4, and 5 are found in sub-Saharan Africa whereas genotypes 3 and 6 are detected with extremely high diversity in South East Asia. This suggests that these geographical areas could be the origin of the different HCV genotypes. The emergence of different HCV genotypes in North America and Europe and other non-tropical countries appears to represent more recent epidemics introduced from the sites of the original HCV endemics **(*Simmonds, 2001 and Biggar et al., 2006*).** HCV-4 is considered a major cause of chronic liver disease and cirrhosis, which leads to liver failure and is an important cause of hepatocellular carcinoma. Because of these complications, extended cirrhosis during chronic infection is a primary cause of liver transplantation in these regions. Although HCV-4 is the cause of approximately 20% of the 170 million cases of chronic hepatitis C (CHC) in the world, it has not been the subject of widespread research **(*Khattab et al., 2011*).**

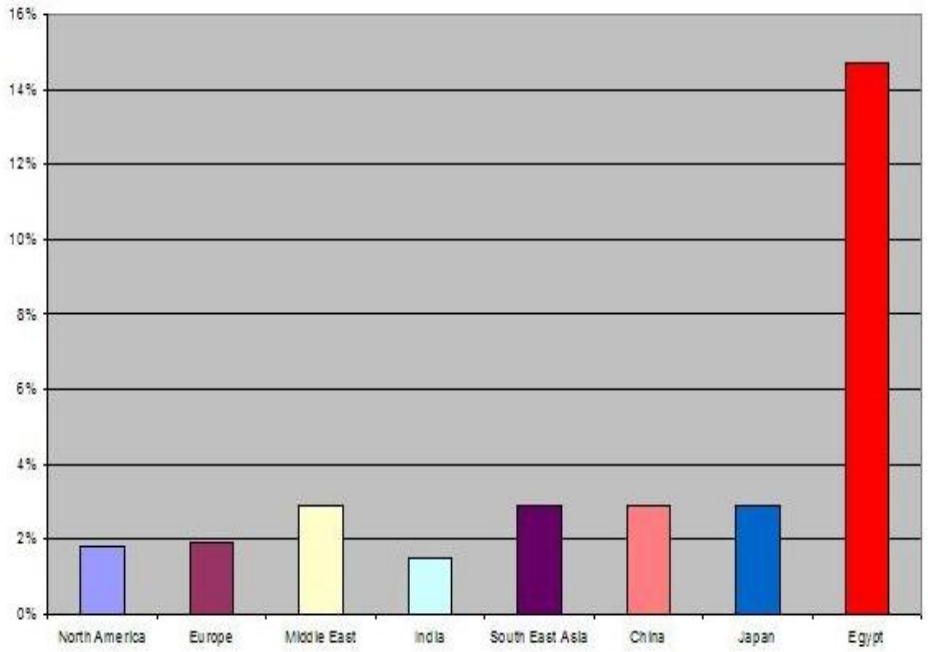

Figure1: Prevalence of hepatitis C virus in Egypt compared to other countries in the world ***(Mohamoud et al., 2013).***

1.3 Viral Structure:

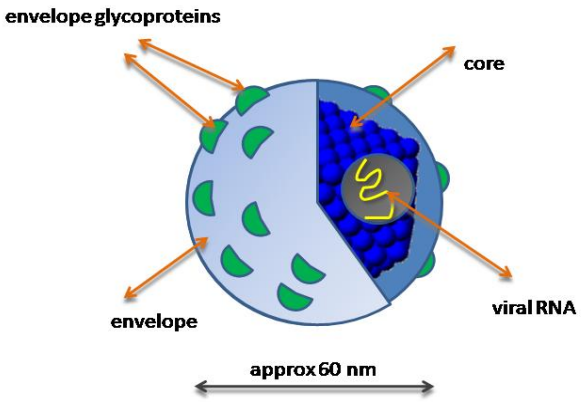

Figure 2: Structure of Hepatitis C Virus ***(Khan et al., 2014).***

HCV particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further

encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope ***(Op De Beeck and Dubuisson, 2003 and Khan et al., 2014).***

1.4 Molecular Virology:

The genomic RNA of HCV serves as messenger RNA (mRNA) for the translation of viral proteins. The linear molecule contains a single open reading frame (ORF) coding for a precursor polyprotein of approximately 3000 amino acid residues. During viral replication the polyprotein is cleaved by viral as well as host enzymes into three structural proteins (core, E1, E2) and seven nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). An additional protein (termed F [frame shift] or ARF [alternate reading frame]) is predicted as a result of ribosomal frame-shifting during translation within the core region of the genomic RNA. The structural genes encoding the viral core protein and the viral envelope proteins E1 and E2 are located at the 5' terminus of the open reading frame followed downstream by the coding regions for the non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B as shown in Figure 3. The structural proteins are essential components of the HCV virions, whereas the non-structural proteins are not associated with virions but are involved in RNA replication and virion morphogenesis **(*Walewski et al., 2001, Xu et al., 2001, Varaklioti et al., 2002 and Dubuisson, 2007).***

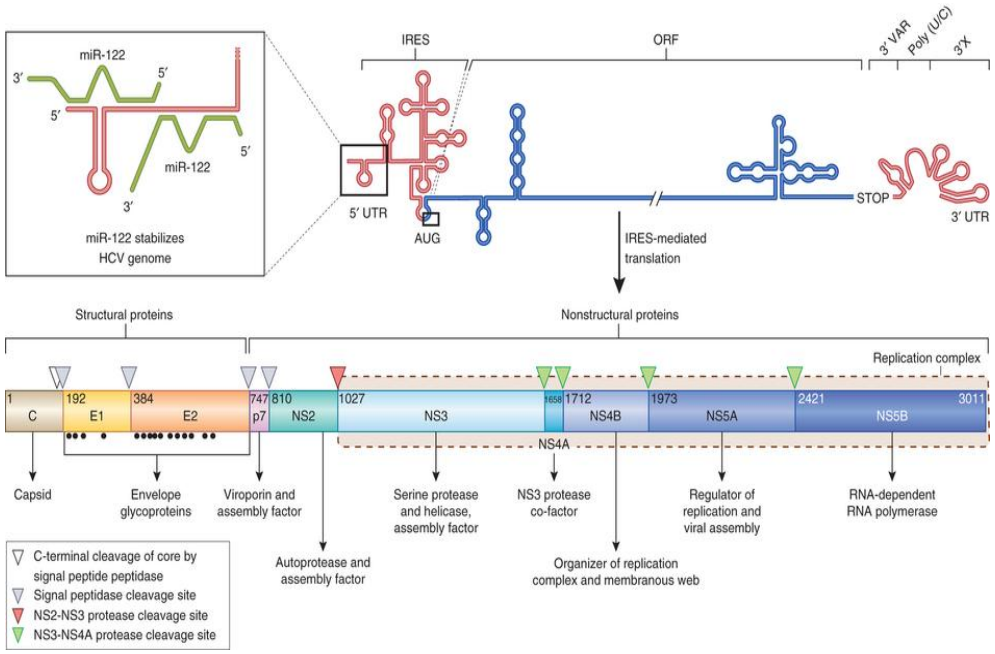

Figure 3: The HCV genome and polyprotein processing
(Scheel and Rice, 2013)

1.5 Viral Lifecycle:

The lifecycle of HCV as illustrated in Figure 4 consists of the following steps:

1.5.1 Adsorption and viral entry

Binding to and entry of HCV into hepatocytes is a very complex multistep process and more host factors involved in that process have been identified over the last 15 years. The first candidate as receptor for HCV was the tetraspanin CD81 **(*Pileri, 1998*)**. The E1-E2 complex is involved in adsorption of the virus to its putative receptors tetraspanin CD81 and low-density lipoprotein receptor (LDLR) inducing fusion of the viral envelope with the host

cell plasma membrane **(*Flint et al., 1999 and Helle and Dubuisson, 2008*).** The multi-step procedure of HCV cell entry was shown to be even more complex since a cellular factor termed claudin-1 (CLDN1) has been identified as being involved in this process **(*Evans et al., 2007*).** Some human cell lines were not susceptible to HCV infection despite expressing The human scavenger receptor class B type I (SR-BI), CD81, and CLDN1 indicating that other cellular factors must be involved in viral entry **(*Evans et al., 2007*).** A cellular four-transmembrane domain protein named occludin (OCLN) was identified to represent an additional cellular factor essential for the susceptibility of cells to HCV infection **(*Liu et al., 2009 and Ploss et al., 2009*)**. It has been shown that two receptor tyrosine kinases (RTKs) and the Niemann–Pick C1-like 1 (NPC1L1) cholesterol uptake receptor are cellular cofactors for HCV entry into hepatocytes **(*Lupberger et al., 2011 and Sainz et al., 2012*).**

1.5.2 Uncoating, translation and post-translational processes

After entry of the viral genome into the host-cell cytoplasm, the virus undergoes an uncoating process to expose the viral genome to host-cell machinery. The viral genome is then translated in preparation for viral replication. The 5' nontranslated region of the HCV genome contains an internal ribosome entry site (IRES) that permits ready access of the viral genome to the host translation machinery for viral-protein synthesis **(*Jackson and Kaminski, 1995).***

Post-translational cleavages lead to at least 10 different protein products which include: the structural proteins (the virus core and the envelope proteins E1 and E2) and the nonstructural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) **(*Moradpour et al., 2002*).**

1.5.3 Viral replication

Replication of HCV involves several steps. The virus replicates mainly in the cytoplasm of hepatocytes of the liver, where it is estimated that daily each infected cell produces approximately fifty virions (virus particles) with a calculated total of one trillion virions generated. The virus may also replicate in peripheral blood mononuclear cells, potentially accounting for the high levels of immunological disorders found in chronically infected HCV patients ***(Bartenschlager and Lohmann, 2000).***

The virus replicates on intracellular lipid membranes ***(Dubuisson et al., 2002).*** The endoplasmic reticulum in particular is deformed into uniquely shaped membrane structures termed membranous webs. These structures can be induced by sole expression of the viral protein NS4B ***(Egger et al., 2002).*** The core protein associates with lipid droplets (LDS) and utilizes microtubules and dyneins to alter their location to a perinuclear distribution ***(Boulant et al., 2008).***

1.5.4 Viral assembly and release

There is a limited amount of data available on the later stages of the HCV life cycle ***(Lindenbach et al., 2005, Wakita et al., 2005***

and Zhong et al., 2005). The viral assembly takes place within the endoplasmic reticulum ***(Gastaminza et al., 2008)*** and that LDS are involved in particle formation ***(Moradpour et al., 1996, Barba et al., 1997, Miyanari et al., 2007, Shavinskaya et al., 2007 and Appel et al., 2008).*** The Core protein, LD-associated NS5A interacting with apolipoproteinE (apoE) seems to play a key role in the formation of infectious viral particles ***(Appel et al., 2008 and Benga et al., 2010).*** The secreted HCV particles have a characteristic low density, which suggests that the virus associates with lipoproteins for viral release. The association of HCV with lipoproteins might also protect the secreted virus from the host's immune response (***Gastaminza et al., 2008***).

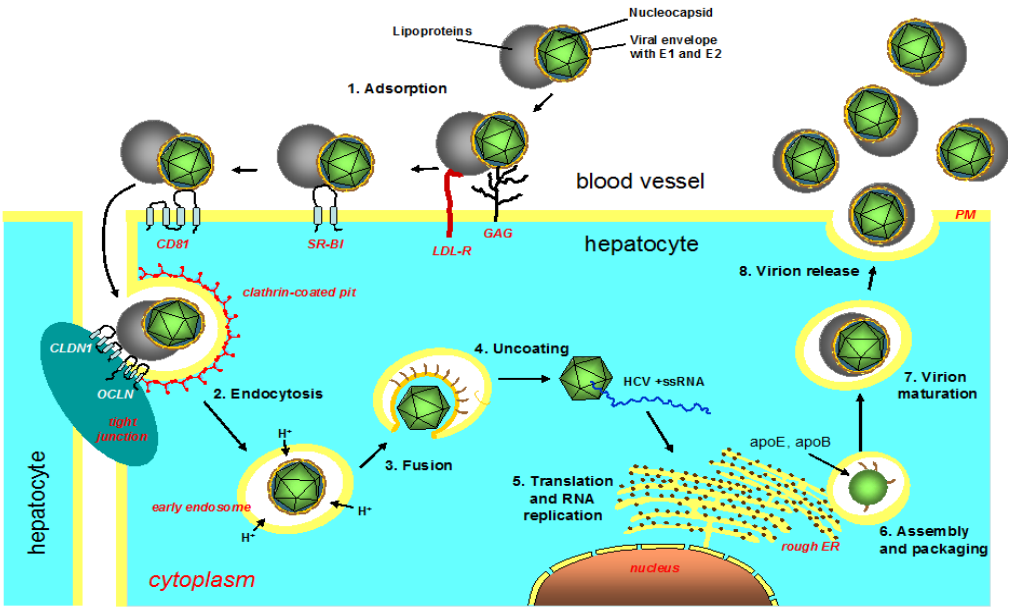

Figure 4: Viral Cycle ***(Pawlotsky et al., 2007).***

1.6 Routes of Transmission:

1.6.1 Injection drug use

Injection drug use has been the most commonly identified source of acute HCV infection. HCV infection also has been associated with a history of intranasal cocaine use, presumably due to blood on shared straws or other sniffing paraphernalia **(*Schmidt et al., 2011 and Rockstroh et al., 2012*).**

1.6.2 Blood transfusion

In the past, blood transfusion or use of other blood products was a major risk factor for transmission of HCV. In some historic cohorts 10% or more of patients who received blood transfusions were infected with hepatitis C **(*Alter et al., 1989*).** However, blood donor screening for HCV since the early 1990s has nearly eliminated this transmission route. Blood donors are screened for anti-HCV antibodies and HCV RNA – at least in developed countries **(*Pomper et al., 2003*).**

In cohorts of multiply transfused patients such as hemophilia, over 90% of patients were infected with hepatitis C in the past **(*Francois et al., 1993*).** Since the use of routine inactivated virus (eg, heat inactivation or pasteurization) or recombinant clotting factors, new cases of hepatitis C infection have become uncommon in these patients ***(Horowitz 1991 and Mannucci et al., 1992).***

1.6. 3 Sexual or household contact

In prospective cohorts of monogamous, heterosexual couples, there was a long-term transmission risk of 0.01% or lower **(*Vandelli et al., 2004*).**

Factors that may increase the risk of HCV infection include greater numbers of sex partners, history of sexually transmitted diseases, and not using a condom. Very often it is difficult to rule out the possibility that transmission results from risk factors other than sexual exposure. Outbreaks of cases of acute hepatitis C in several cities in Europe and the United States among men who have sex with men (MSM) have focused attention on sexual transmission of HCV **(*Boesecke et al., 2012 and Rockstroh, 2012).***

There is clear evidence that unprotected sex can transmit HCV. Unprotected anal sex, fisting, having many sex partners in a short time period, a concomitant sexually transmitted disease including HIV and use of recreational drugs were identified as risk factors **(*Danta et al., 2007 and Schmidt et al., 2011*).**

1.6.4 Organ transplantation

Transplant recipients who receive organs from HCV-positive donors have a high risk of acquiring HCV infection. Therefore, most transplant organizations have developed strategies for screening and selective utilization of organs from anti-HCV positive donors **(*Strong et al., 2010*).**

1.6.5 Hemodialysis

Patients who participate in chronic hemodialysis programs are at increased risk for hepatitis C **(*Fissell et al., 2004*).** Most dialysis patients are already infected when they present with end-stage renal disease, but acute infection is common in dialysis centers ***(Bergman et al., 2005, Finelli et al, 2005 and Izopet et al., 2005).***

1.6.6 Perinatal transmission

HCV can be transmitted from an infected mother to her child in the perinatal period (vertical transmission). When no preventive measures are taken to prevent transmission of HCV, human immunodeficiency virus (HIV), or hepatitis B virus (HBV), vertical transmission of HCV occurs relatively less frequently than with HIV or HBV. Caesarean section has not been shown to reduce the transmission risk. There is no evidence that breastfeeding is a risk for infection among infants born to HCV-infected women. Early diagnosis of infection in newborns requires HCV RNA testing since anti-HCV antibodies are passively transferred from the mother **(*Conte et al., 2000, Resti et al., 2003, Mast et al., 2005 and Polis et al., 2007*).**

1.6.7 Needle stick injury

There is some risk of HCV transmission for healthcare workers after unintentional needle stick injury or exposure to other sharp objects **(*MMWR, 2001*).** However, data are divergent and

figures ranging from 0 to 10% can be found. Hollow-bore needle exposures typically involve a larger inoculum than solid-bore needle exposures and thus, a greater risk of transmission. Exposure of HCV to intact skin has not been associated with HCV transmission **(*Mitsui et al., 1992 and Sarrazin et al., 2010*).**

1.6.8 Other rare transmission routes

Rare sources of percutaneous transmission of HCV are contaminated equipment used during medical procedures, procedures involved in traditional medicine (eg, scarification, cupping), tattooing, and body piercing. All these routes bear the potential of transmitting HCV. In addition, transmission via these routes is so rare that persons with exposure are not at increased risk for acquiring HCV ***(Haley and Fischer 2001 and Nishioka et al., 2002).***

Hepatitis C is not spread through breast milk, food or water or by casual contact such as hugging, kissing and sharing food or drinks with an infected person ***(WHO, 2011).***

1.7 Natural History:

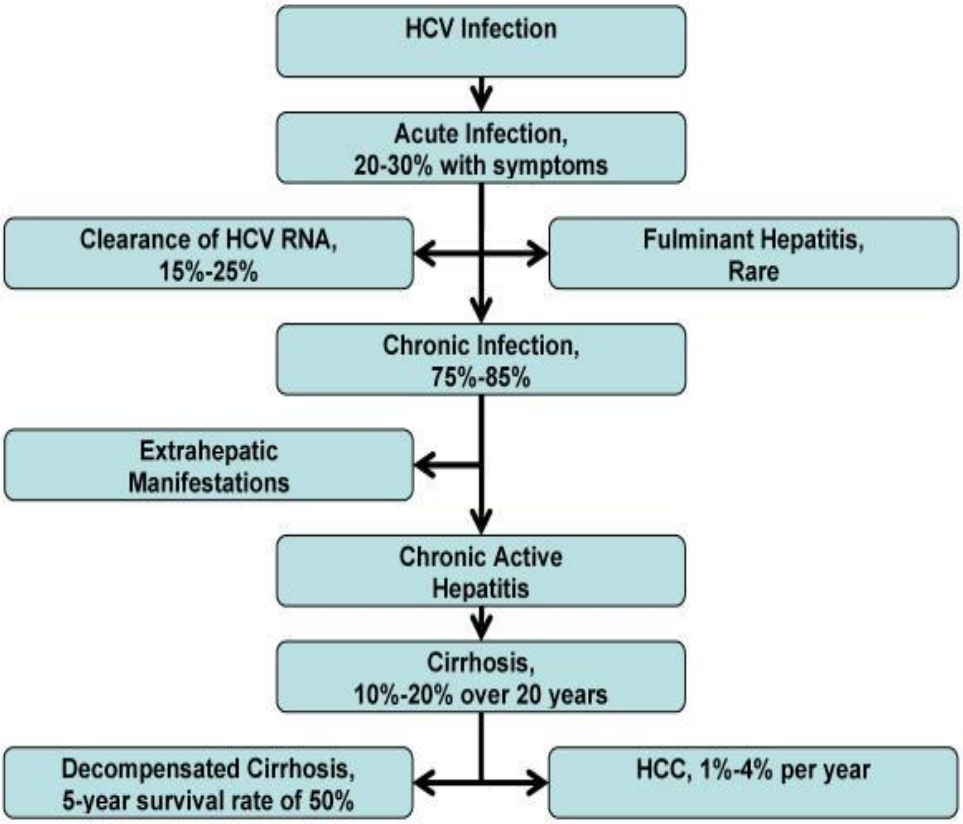

Figure 5: Natural History of HCV Infection ***(Chen and Morgan, 2006*).**

1.7.1 Acute hepatitis C virus infection

Acute hepatitis C infection is infrequently diagnosed because the majority of acutely infected individuals are asymptomatic. In the transfusion setting, where acute onset of HCV infection has been best documented, 70% to 80% of cases were asymptomatic ***(McCaughan et al., 1992).*** About 20% to 30% of adults with acute HCV infection may develop clinical symptoms. The symptomatic onset ranges from 3 to 12 weeks after exposure ***(Alter and Seeff, 2000 and Thimme et***

al., 2001). Symptoms may include malaise, weakness, anorexia, and jaundice ***(Farci et al., 1996).***

The symptoms can last several weeks and subside as serum alanine aminotransferase (ALT) and HCV RNA levels decline. The fluctuating nature of the infection can be seen in 10–15% of the subjects with marked variation in levels of ALT and aspartate aminotransferase (AST) in association with marked changes in HCV RNA levels including periods of HCV RNA negativity followed by reappearance of HCV RNA. In most patients, fluctuations are present only during the first 24 weeks after exposure; however, these may continue beyond 24 weeks **(*Loomba et al., 2011*).**

1.7.2 Chronic HCV infection

Chronic hepatitis C is marked by the persistence of HCV RNA in the blood for at least 6 months after onset of acute infection. HCV is self-limiting in only 15%-25% of patients in whom HCV RNA in the serum becomes undetectable and ALT levels return to normal. Approximately 75%-85% of infected patients do not clear the virus by 6 months, and chronic hepatitis develops ***(Chen and Morgan, 2006).***

1.7.2.1 Risk factors for developing chronic HCV infection

The rate of chronic HCV infection is affected by many factors, including:

1. ***Age :***

In the National Health and Nutrition Examination Survey (NHANES) study, the chronicity rate was estimated at 30% in subjects below the age of 20 years, and 76% for those older than 20 years ***(Alter et al., 1999).***

The later study suggested that persons with HCV infection at younger age, less than 25 years, are less likely to have CHC than those infected at older ages ***(Chen and Morgan, 2006).***

2. ***Gender :***

The NHANES study and the Dionysos study had similar rates of HCV chronicity among both men and women ***(Alter et al., 1999 and Bellentani and Tiribelli, 2001).***

3. ***Ethnicity :***

According to the NHANES study, only 14% of black men were able to spontaneously clear the virus compared to 32% of whites **(*Howell et al., 2000*).** Interestingly, African-Americans and Asians with HCV have a 2-fold and 4-fold increased risk, respectively, of developing hepatocellular carcinoma (HCC) when compared to Caucasians with HCV **(*Nguyen et al., 2004*).**