INTRODUCTION

Estimates place the number of HCV infected individuals at approximately 170 million, representing nearly 3% of the world's population.[1] The natural course of HCV infection has two distinct virologic outcomes: acute infection with subsequent clearance and viral persistence leading to chronic infection. Unfortunately, only 30% of patients naturally clear HCV, with the majority remaining persistently infected. Active HCV replication occurs for decades in these individuals, leading to chronic hepatitis, progressive fibrosis, cirrhosis and hepatocellular carcinoma. HCV infection and associated pathologies are responsible for an estimated 250,000 deaths a year worldwide. [2]

STRUCTURAL BIOLOGY OF HCV

HCV is the sole member of the *Hepacivirus* genus of the *Flaviviridae* family of small, enveloped, single strand positive sense RNA viruses. The 9.6 kb viral genomic RNA contains a single open reading frame flanked by highly structured non-translated regions (NTRs) (Figure 1). The genome of HCV encodes at least ten proteins, of which three are structural proteins (core, E1, E2) and six non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). They are synthesized as polyprotein which is cleaved into distinct products by both cellular and virally encoded proteases in the endoplasmic reticulum.[3]

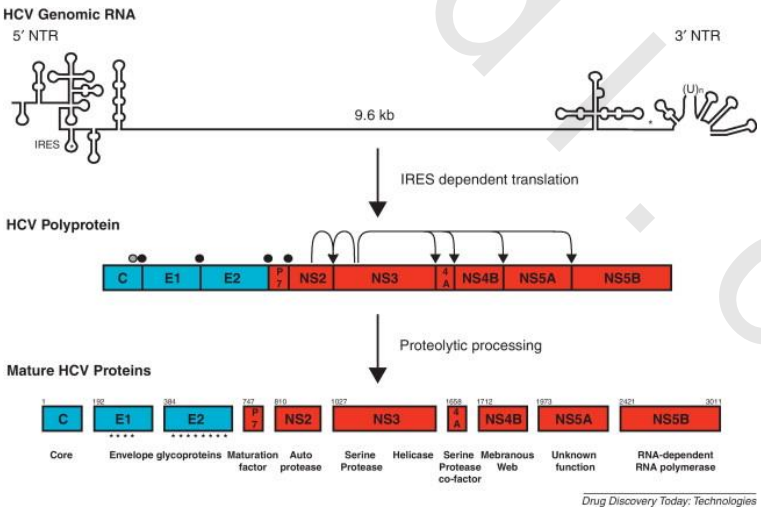

Figure 1: HCV genomic organization, polyprotein processing and functions of mature HCV proteins.

HCV GENOME STRUCTURE AND ORGANIZATION (Figure 2)

5’ untranslated region:

The HCV 5'UTR contains 341 nt located upstream of the ORF translation initiation codon. It is the most conserved region of the genome. the HCV 5'UTR is not capped and folds into a complex secondary RNA structure forming, together with a portion of the core-coding domain, an internal ribosome entry site (IRES) that mediates direct binding of ribosomal subunits and cellular factors and subsequent translation. The HCV IRES has the capacity to form a stable pre-initiation complex by directly binding the 40S ribosomal subunit without the need of translation initiation factors, an event that likely constitutes the first step of HCV polyprotein translation.[3, 4]

3’ untranslated region:

The 3'UTR contains approximately 225 nt. It is organized in three regions including,from 5' to 3', a variable region of approximately 30-40 nt. a long poly(U)-poly(U/ UC) tract, and a highly conserved 3'-terminal stretch of 98 nt (3'X region). The 3'X region and the 52 upstream nt of the poly(U/C) tract were found to be essential for RNA replication, whereas the remaining sequence of the 3'UTR appears to enhance viral replication.[3, 4]

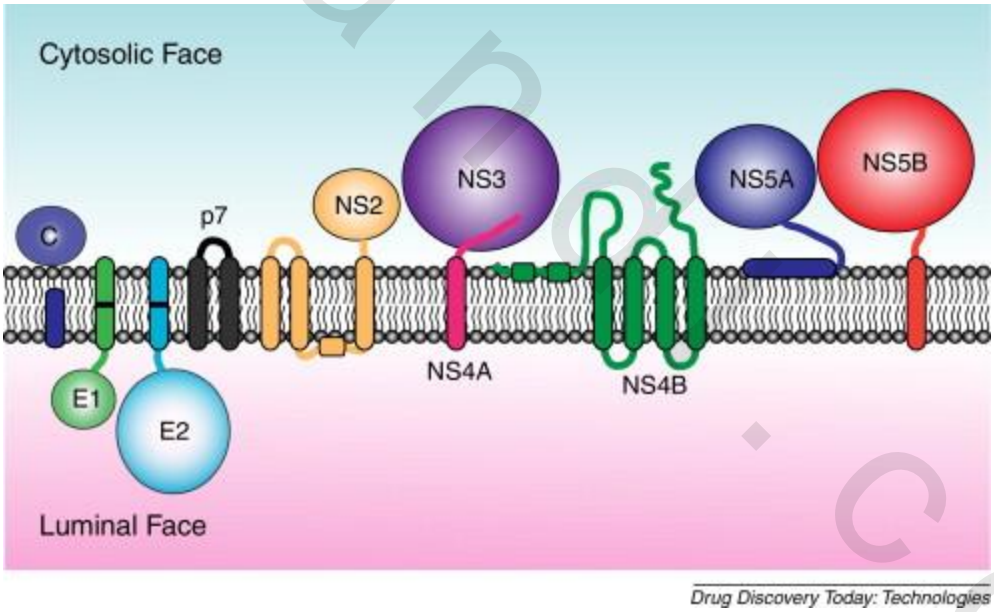

Figure 2. Topological arrangements of HCV proteins

CHARACTERISTICS AND FUNCTIONS OF HCV PROTEINS (Table 1)

Core:

The HCV core protein is a small (19–21 kDa), basic, RNA-binding protein that is probably the major protein component of the viral nucleocapsid. Core is removed from the viral polyprotein by the host signal peptidase via a cleavage at the C-terminus of the core coding sequence, yielding an immature form of the protein tethered to the membrane. Subsequent cleavage by signal peptide peptidase in the interior of the ER membrane releases the mature form of the core, and generates the signal sequence of the E1 protein that follows core in the viral polyprotein.[3, 5, 6]

In addition to its role in viral capsid formation, the core protein has been suggested to directly interact with a number of cellular proteins and pathways that may be important in the viral replication cycle. The HCV core protein has pro- and anti-apoptotic functions, stimulates hepatocyte growth in Huh-7 cell line by transcriptional upregulation of growth-related genes, and has been implicated in tissue injury and fibrosis progression. The HCV core protein could also regulate the activity of cellular genes, including *c-myc* and *c-fos*, and alter the transcription of other viral promoters. It induces hepatocellular carcinoma when expressed in transgenic mice. It could also induce the formation of lipid droplets and may play a direct role in steatosis formation.[3, 5, 6]

E1 and E2

The HCV glycoproteins, the outermost components of the infectious virus particle, consist of two distinct polypeptide chains: E1 (~35 kDa) and E2 (~70 kDa). These proteins form a non-covalent heterodimeric complex in cells, and this complex probably represents the functional 'spikes' on the surface of the virus particle.[3, 7, 8]

E1 and E2 are both processed from the viral polyprotein by the host signal peptidase, and both are oriented as type I transmembrane proteins, with large N-terminal ectodomains, and short luminal C-terminal regions. This similar topological arrangement of two proteins that are encoded adjacent to one another in the viral polyprotein is rather unusual. Indeed, the highly unique nature of the E1 and E2 transmembrane regions adopt a peculiar hairpin conformation before signal peptidase cleavage, followed by a rearrangement post-cleavage to generate the proper protein orientation. The sizes listed for these proteins are approximate values as both proteins are heavily modified by N-linked glycosylation, with as many as 6 sites on E1, and perhaps 11 on E2. These sites appear to be important for the proper folding of the glycoproteins, and may play a role in receptor interactions. E2 is primarily responsible for receptor interactions during virus entry, and E1 seems to function as a scaffold for the proper orientation and stability of E2. Once processed and matured, the E1 and E2 heterotypic complexes are retained in the ER via signals in the E1 and E2 transmembrane region, although a small amount of these proteins can also be found on the cell surface. As budding of virions occurs into the lumen of internal membrane compartments, the retention of these proteins in the ER makes sense. [3, 9, 10]

E2 contains hypervariable regions with aa sequences differing up to 80% between HCV genotypes and between subtypes of the same genotype. Hypervariable region 1 (HVR1) contains 27 aa and is a major (but not the only) HCV neutralizing epitope. Despite the HVR1 sequence variability, the physicochemical properties of the residues at each position and the overall conformation of HVR1 are highly conserved among all known HCV genotypes, suggesting an important role in the virus replication cycle. E2 plays a crucial role in the early steps of infection. Viral attachment is thought to be initiated via E2 interaction with one or several components of the receptor complex. Because HVR1 is a basic region with positively charged residues located at specific sequence positions, it can theoretically interact with negatively charged molecules at the cell surface. This interaction could play a role in host cell recognition and attachment, as well as in cell or tissue compartmentalization. Less is known about E1, but it is thought to be involved in intra-cytoplasmic virus-membrane fusion.[3, 9, 11]

p7

p7 is a small (7 kDa), hydrophobic protein which contains possible endoplasmic reticulum (ER) retention signals, and belongs to the family of viroporins: small virally encoded membrane proteins that oligomerize to form channels and could act as calcium channels. Although crucial for late phases of assembly as well as the release of infectious virus, p7 is not required for HCV RNA replication. A polytopic membrane protein that is thought to cross the membrane twice, with both N and C termini oriented toward the lumen of the ER, p7 monomers have been shown to assemble into hexamers or heptamers in lipid bilayers and form cation-selective ion channels.[3, 12, 13]

NS2

The HCV NS2 protein is a 217-amino acid long (21 kDa), membrane-associated cysteine-protease essential for HCV replication in the context of the complete viral genome, as the protease activity of NS2 auto-catalytically cleaves the NS2-3 junction in *cis*, thereby liberating the NS3 protease that cleaves the subsequent downstream sites in the polyprotein. NS2 also plays an important but poorly defined role in the production of infectious virus, although its catalytic active site is not required for this process. [3, 13, 14]

NS3/NS4A

NS3-NS4A is a large (69 kDa), multi-functional, complex enzyme, with NS3 containing the functional enzymatic activities, and NS4A functioning as a co-factor for these functions as well as a membrane anchor to attach NS3 to cellular membranes. NS3 is a hydrophobic protein that contains two distinct functionalities: the amino terminal region of the protein functions as a serine protease, while the carboxyl terminal region contains an RNA helicase. Both of these activities are modulated by the presence of the NS4A co-factor, and are essential for RNA replication. Like several other non-structural proteins, NS3/4A also plays an undefined role in virus particle formation. The serine protease domain is the major viral polyprotein-processing enzyme, cleaving the NS3/NS4A junction in *cis*, and all the remaining downstream junctions in the polyprotein in *trans*. Not surprisingly, the NS3-NS4A protease is one of the most popular viral targets for anti-HCV therapeutics. [3, 13]

In addition to cleaving the viral polyprotein, NS3 has emerged as an important protein for the viral escape of the innate immune system. NS3 cleaves the cellular signaling molecules, resulting in a 'short-circuiting' of the signal transduction pathways to the transcription factors IRF-3 and NFκβ.[3, 13]

The NS3 helicase is capable of ATP-dependent unwinding of double stranded RNA, or highly structured single stranded RNA in a 3′ to 5′ direction. Although the helicase domain alone is enzymatically active, full activity requires the NS3 protease domain, as the protease assists in proper positioning of the RNA substrate. The natural substrate for NS3 in the HCV infected cell is not known, but it has been hypothesized that the helicase unwinds double stranded replicative intermediates to expose essential RNA replication elements, or unpairs the structures on the NTRs of the genome to allow access to the polymerase during replication. As the helicase is an essential enzymatic activity for genome replication, one might expect this to be a prime target for anti-viral compound development, but progress in this area has been relatively slow.[3, 13]

NS4B

The HCV NS4B protein is a 27 kDa integral membrane, highly hydrophobic protein, and its expression alone is sufficient to trigger formation of the membranous web (MW) – a structure probably derived from the ER and believed to be the site of HCV RNA replication. NS4B is believed to contain an N-terminal tail that is translocated post-translationally to the ER lumen thereby targeting NS4B to intracellular membranes.[3, 4, 7, 13]

Additional putative properties include inhibition of cellular synthesis, modulation of HCV NS5B RdRp activity , transformation of NIH3T3 cell lines, and induction of interleukin 8.[3]

NS5A

NS5A is a large (56–58 kDa), hydrophilic phosphoprotein protein of unknown function. Despite not having a clear role in the HCV replication cycle, NS5A is essential for both RNA replication and virus assembly, and has recently emerged as a potential regulator of these events, presumably functioning through phosphorylation of the protein. [3, 15]

Multiple functions have been assigned to NS5A based on its interactions with cellular proteins. NS5A appears to play a role in interferon resistance by binding to and inhibiting PKR, an antiviral effector of interferon. NS5A also bears transcriptional activation functions and appears to be involved in the regulation of cell growth and cellular signaling pathways.[3, 15]

NS5B

The NS5B protein comprises the viral RNA-dependent RNA polymerase and is required for RNA replication. NS5B is a large (68 kDa), hydrophilic protein that is associated with ER derived membranes via a hydrophobic 21 amino acid α-helix at the C-terminus of the protein. The insertion of this sequence into membranes occurs post-translationally, making NS5B a member of the tail-anchored class of membrane proteins. The activity of NS5B is modulated by

several HCV replicase proteins, including NS3, a positive stimulator, and NS4A and NS5A, both negative regulators.[3, 16]

The crystal structure of NS5B revealed that the RdRp has a classical "fingers, palm and thumb" structure formed by its 530 N-terminal aa. Interactions between the fingers and thumb subdomains result in a completely encircled catalytic site that ensures synthesis of positive- and negative-strand HCV RNAs. The RdRp is another important target for the development of anti-HCV drugs.[3, 13, 16]

Frameshift Protein (ARFP)

The F (frameshift) protein or ARFP (alternate reading frame protein) is generated as a result of a -2/+1 ribosomal frameshift in the N-terminal core-encoding region of the HCV polyprotein. Antibodies to peptides from the F protein were detected in chronically infected patients, suggesting that the protein is produced during infection. However, the exact translational mechanisms governing the frequency and yield of the F protein during the various phases of HCV infection are completely unknown. Thus, the role of F protein in the HCV lifecycle remains enigmatic but it was proposed to be involved in viral persistence.[3]

Table 1: Functions and molecular weight of HCV proteins.

HCV protein	Function	Apparent molecular weight (kDa)
Core	Nucleocapsid	23 (precursor) 21 (mature)
F/ARF[a]-protein	?	16-17
E1	Envelope Fusion domain?	33-35
E2	Envelope Receptor binding Fusion domain?	70-72
p7	Calcium ion channel (viroporin)	7
NS2	NS2-3 autoprotease	21-23
NS3	Component of NS2-3 and NS3-4A proteinases NTPase/helicase	69
NS4A	NS3-4A proteinase cofactor	6
NS4B	Membranous web induction	27
NS5A	RNA replication by formation of replication complexes	56 (basal form) 58 (hyperphosphorylated form)
NS5B	RNA-dependant RNA polymerase	68
[a] Frameshift/ alternate reading frame		

HCV Replication Cycle:

The virus attaches to specific receptors (CD81, SR-B1, DC-SIGN, L-SIGN, LDL-R, asialoglycoproteins and glycosaminoglycans), then fusion, uncoating, protein and RNA synthesis starts. Translation initiation is mediated by an internal ribosome entry site (IRES) located in the 5′ NTR, leading to the synthesis of a roughly 3000 amino acid polyprotein that is cleaved by both viral and host proteases to yield 10 mature viral proteins. These include the structural proteins (Core, E1 and E2), and the non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). A subset of the non-structural proteins localize to a site of ER expansion termed the membranous web (MW) that is created by the NS4B protein, and assemble into an RNA replication complex. [7, 11]

The replication complex requires NS3, NS4A, NS5A, and NS5B, as well as several host proteins, to copy the viral genome into a minus sense replicative intermediate and back into new progeny genomic RNAs. The viral core protein localizes to cytoplasmic lipid droplets, which are believed to be the site of nucleocapsid assembly. At some point in the replication cycle, the newly made viral RNA is brought close to the lipid droplets bearing the core protein, and nucleocapsid assembly occurs. These particles associate with the viral glycoproteins that are retained and matured in the endoplasmic reticulum (ER), and budding of progeny virions occurs into internal membrane compartments. These particles then mature and are released from the cell via the host secretory pathway, thereby completing the viral lifecycle.[7, 11]

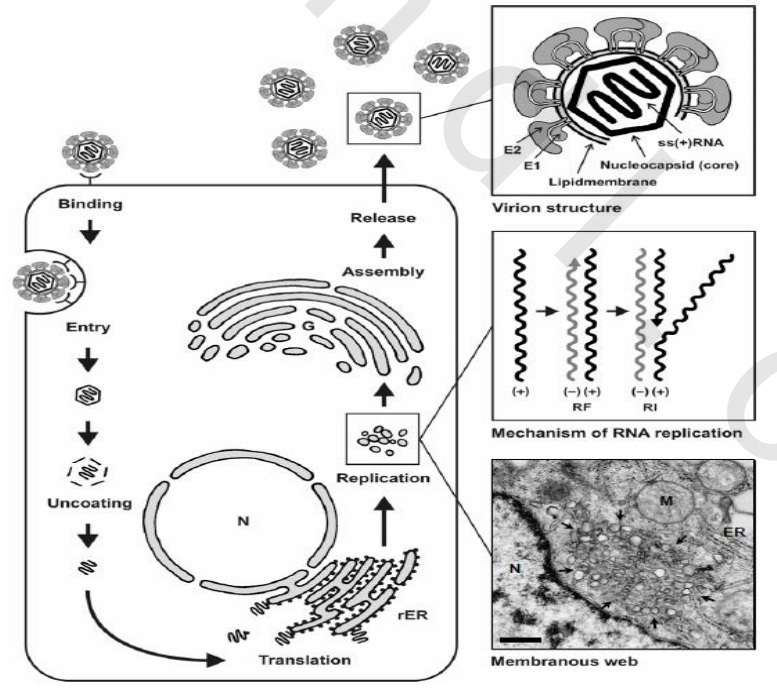

Figure 3: HCV Replication Cycle

Immunopathogenesis of Hepatitis C:

Unlike other hepatitis viruses, the hepatitis C virus is more likely to cause clinically inapparent, chronic infection in persons who are otherwise considered immunocompetent. Thus, the virus is capable of overcoming an efficient immune response of the host.[11]

Components of Antiviral Immune Response: (Figure 4)

The mechanisms whereby HCV overcomes immune response and establishes persistent infection are currently undefined. It is well known that the specific immune response to any viral infection is primed by macrophages and dendritic cells that present viral proteins to B cells, helper T cells, and cytotoxic T cells . In many viral infections, B cells produce antibodies that can clear circulating virus and protect from reinfection. Through specific T-cell receptors on the cell surface, helper T cells recognize viral peptides that are derived from phagocytosed and proteolytically cleaved HCV proteins and are presented in the context of class II MHC molecules. On activation of their specific T-cell receptors, HCV-specific helper T cells assist with activation and differentiation of B cells as well as induction and stimulation of virus-specific cytotoxic T cells. Most of these effects are mediated by different sets of immunoregulatory Th1 (interferon- λ and interleukin- 2) or Th2 (interleukin-4, interleukin-5, and interleukin-10) cytokines. In the context of class I MHC molecules, CD8-positive cytotoxic T cells recognize HCV peptides that are synthesized and processed in infected cells. This encounter can lead to lysis of virus-infected cells. Together with helper T cells, cytotoxic T lymphocytes may also secrete cytokines, such as interferon-g and tumor necrosis factor-a, that inhibit replication and gene expression of several viruses.[11, 17, 18]

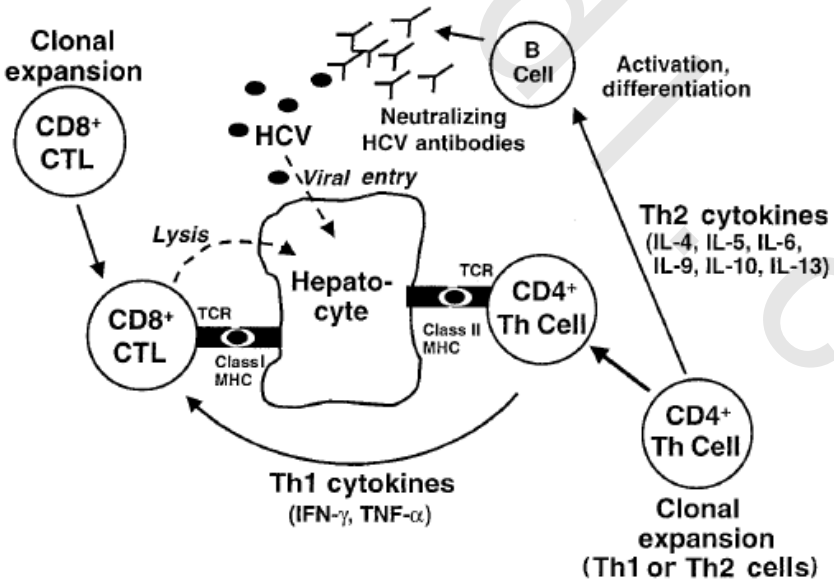

Figure 4: Components of HCV antiviral immune response

Humoral Immune Response:

Hepatitis C virus can establish persistent infection despite an active humoral and cellular immune response that is generally targeted against all viral proteins. The virus may escape from the humoral immune response if the kinetics of infection and viral replication do not allow complete neutralization of the virus by HCV-specific antibodies after primary infection. Although virus-specific antibodies may interfere with viral entry into host cells and opsonize the virus for elimination by macrophages, they cannot eliminate HCV from infected cells. In addition, HCV has a high mutation rate, especially in the hypervariable region of the envelope proteins that can be recognized by neutralizing antibodies. Several studies have demonstrated that the humoral immune response can select HCV variants with sequence changes that allow escape from antibody recognition. Thus, progression to persistent HCV infection is most likely a multifactorial process that depends on multiple aspects of virus– host interaction.[19, 20]

Cellular Immune Response:

The cellular immune response probably plays an important role in the outcome of HCV infection because of its ability to recognize and eliminate virus from infected cells. Most studies have concentrated on the antigen-specific immune response that is mediated by CD4-positive helper T cells and CD8-positive cytotoxic T cells. Because chronic rather than acute infection is diagnosed in most patients, immunologic studies have been performed on patients with persistent infection who could not clear HCV. Only a few studies have analyzed the cellular immune response during the acute phase of infection.

The cellular response against HCV could be interfered with in several ways. First, HCV elicits only a weak T-cell response in patients who develop chronic infection. In the blood of patients with chronic hepatitis C, the frequency of cytotoxic T-cell precursors that are specific for individual HCV peptides is much lower than the frequency of T cells that recognize an influenza virus peptide as a recall antigen or peptides of other viruses that can be cleared, such as cytomegalovirus . The reasons for this relative weakness of the cellular immune response are not known. Certainly, general immune tolerance or immunosuppression is not the cause of persistent HCV infection, because most chronically infected patients display normal immune responses against other viral agents. [17, 21, 22]

The emergence of viral mutants or quasi-species with sequence variations in T-cell epitopes may contribute to the apparent ineffectiveness of cell-mediated immune response. There is also increasing evidence that several HCV proteins, such as core, E2, and NS5A, interfere with the immune response. Furthermore, infected hepatocytes, which lack co-stimulatory molecules, may be relatively inefficient in priming the immune system, and the liver has been proposed as the major site where activated T cells are destroyed. Finally, the cellular immune response is a double-edged sword. An immune response that is ineffective in clearing HCV infection may be more harmful to the liver, causing chronic inflammation, hepatocellular injury, and, over several decades, liver fibrosis and cirrhosis.[20]

Progression to persistent infection and the immunologic mechanisms of liver injury are the consequence of complicated interactions between the virus and host. Identification of immunologic correlates of viral clearance may contribute to the development of an effective vaccine and better therapy for HCV infection.[20]

Evasion of the immune response by quasispecies variation:

HCV as a consequence of its sequence variability is present as a pool of viruses presenting different epitopes. Modifications of both B and T epitope patterns during HCV infection have been observed and could contribute to HCV evasion from the immune system. One possible way to escape the humoral response is to have a large diversity of epitopes that can not be neutralized by antibodies.[17, 19, 23]

Several studies have shown that during HCV infection, the HVR1 sequence of E2 became progressively heterogeneous, suggesting that it is a target of selection by antiviral antibodies. Neutralization-escape variants have been isolated during HCV infection. In HCV patients with impaired humoral immune response, the HVR1 has a lower mutation rate compared to immunocompetent individuals, suggesting that mutations in the HVR1 region are the result of selective pressure and that HVR1 contains dominant B epitopes. In HCV-infected patients, antibodies are produced early after infection. If the quasispecies pattern is limited, infection is circumvented and the virus is eliminated. However, if the quasispecies pattern continues to evolve, persistent infection results. Anti-HVR1 antibodies, referred to as 'neutralizing of binding' (NOB) antibodies, are able to bind recombinant E2, HCV viruslike particles or bona fide viral particles. But, there is no definitive proof that they block viral entry. A correlation has been observed between prolonged high NOB titers in patients and natural resolution of chronic hepatitis C, suggesting that they can play a role in viral clearance. However, lack of an efficient model of infection renders the validation of this hypothesis difficult. [17, 19, 23]

These observations suggest that selection of viral variants that cannot be efficiently neutralized by anti-HVR1 antibodies probably contributes to the failure of elimination of HCV, leading to the establishment of a persistent infection.[19]

Epidemiology and Natural History of HCV

After the discovery of the hepatitis C virus in 1989 and its linkage to non-A, non-B hepatitis, HCV was first thought to be an infection of minor importance, affecting selected drug user and blood product recipient populations in developed countries. More than 20 years later, it is now well established that HCV is of global importance, affecting all countries, leading to a major global health problem that requires widespread active interventions for its prevention and control. This is no surprise as the spread of HCV (based on the rate of development of molecular diversity) can be estimated to date back about 500–2000 years.[24, 25]

There is much controversy surrounding the natural history of hepatitis C infection. The rate of chronic HCV infection is affected by a person's age, gender, race, and viral immune response. Approximately 75%-85% of HCV-infected persons will progress to chronic HCV infection, and are at risk for the development of extrahepatic manifestations, compensated and decompensated cirrhosis, and hepatocellular carcinoma (HCC). The rate of progression to cirrhosis is highly variable, and is influenced by several factors, including the amount of alcohol consumption, age of initial HCV infection, degree of inflammation and fibrosis on liver biopsy, HIV and HBV coinfection, and comorbid conditions. An estimated 10%-15% of HCV-infected persons will advance to cirrhosis within the first 20 years. Persons with cirrhosis are at increased risk of developing HCC. An understanding of the natural history of hepatitis C is essential to effectively manage, treat, and counsel individuals with HCV infection (Figure 5).[2, 26]

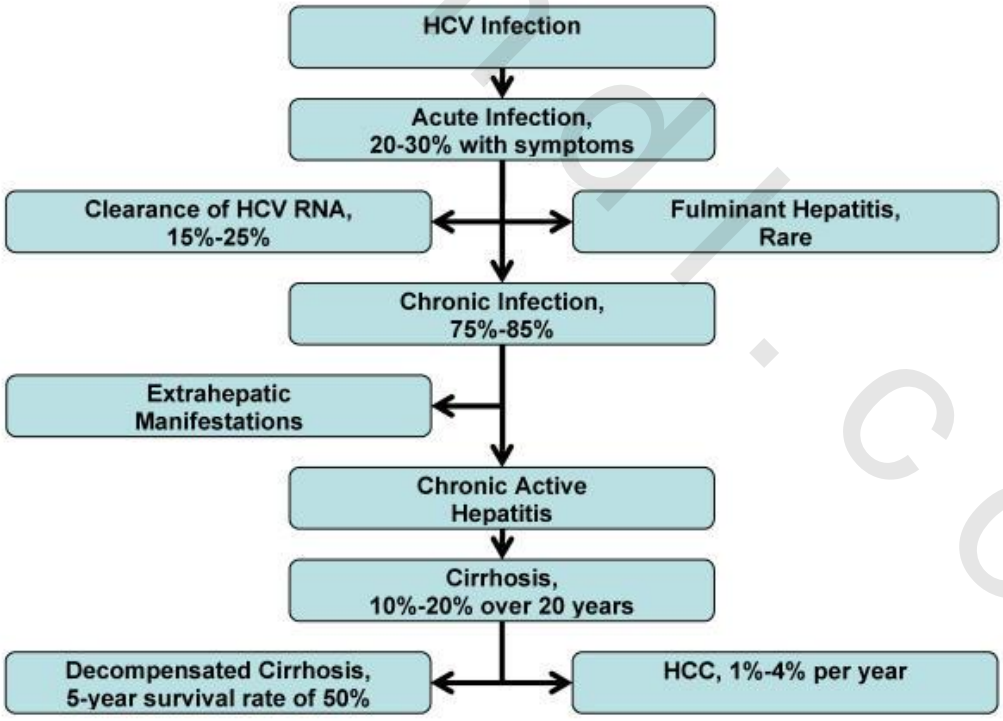

Figure 5: Natural history of HCV

1.Acute Hepatitis C:

Acute hepatitis C infection is infrequently diagnosed because the majority of acutely infected individuals are asymptomatic. 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. Symptoms may include malaise, weakness, anorexia, and jaundice. Serum alanine aminotransferase (ALT) levels, signifying hepatocyte necrosis, begin rising 2 to 8 weeks after exposure, and often reach levels of greater than 10 times the upper limits of normal. HCV RNA can be detected in the serum within 1 to 2 weeks after exposure. The level of HCV RNA rises rapidly during the first few weeks, and then peaks between 105 to 107 IU/ml, shortly before the peak of serum aminotransferase levels and onset of symptoms. In self-limited acute hepatitis C, symptoms can last several weeks and subside as ALT and HCV RNA levels decline. Acute HCV infection can be severe, but fulminant liver failure is rare. The antibody to HCV, as detected by enzyme immunoassay, becomes positive near the onset of symptoms, approximately 1 to 3 months after exposure. Up to 30% of patients will test negative for anti-HCV at onset of their symptoms, making anti-HCV testing unreliable in diagnosis of acute infection. Almost all patients eventually develop the antibody to HCV; however, titers can be low or undetectable in immunodeficient patients. The anti-HCV assay detects greater than 90% of HCV infections after the initial 3 months.[27, 28]

2.Chronic Hepatitis C:

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. The rate of chronic HCV infection is affected by many factors, including the age at time of infection, gender, ethnicity, and the development of jaundice during the acute infection (table 2).[26, 29]

Table 2: Risk factors for chronic Hepatitis C

Risk Factors
Age at time of infection > 25 years
Male gender
No jaundice or symptoms during acute infection
African American race
HIV infection
Immunosuppression

3.Liver Fibrosis:

In the setting of persistent hepatitis C viremia, the rate of progression of liver fibrosis varies widely. There have been extensive studies focusing on the natural course of disease progression from chronic hepatitis C to cirrhosis, HCC, and death. The liver biopsy is the gold standard for the grading and staging of chronic hepatitis C. The activity of liver disease or grade, is gauged by

the number of mononuclear inflammatory cells present in and around the portal areas, and by the number of dead or dying hepatocytes. The structural liver damage, also known as fibrosis or stage, is variable in chronic HCV infection. Fibrosis implies possible progression to cirrhosis. In mild cases, fibrosis is limited to the portal and periportal areas. More advanced changes are defined by fibrosis that extends from one portal area to another, also known as "bridging fibrosis."[26, 30]

4.Cirrhosis

Cirrhosis develops in approximately 10% to 15% of individuals with chronic HCV infection. There are external and host factors that can increase the risk of progression of liver disease. Multiple studies have shown that chronic alcohol use is a major external risk factor for the progression of chronic hepatitis C to cirrhosis and HCC. Host risk factors include older age at time of infection, male gender, the degree of inflammation and fibrosis present on the liver biopsy, coinfection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV), and comorbid conditions such as immunosuppression, insulin resistance, non-alcoholic steatohepatitis, hemochromatosis, and schistosomiasis.[30]

Table 3: Risk factors for development of cirrhosis in HCV infection

Risk Factors
Alcohol consumption (>30 g/day in males, >20 g/day in females)
Age at time of infection > 40 years
Male gender
Degree of inflammation and fibrosis on liver biopsy
Coinfection with HIV or HBV
Comorbid disease

The progression to cirrhosis is often clinically silent, and some patients are not known to have hepatitis C until they present with the complications of end-stage liver disease or HCC. The features of decompensated cirrhosis include the development of ascites, upper gastrointestinal bleeding secondary to varices or portal hypertensive gastropathy, hepatorenal syndrome and hepatic encephalopathy.[2]

Since HCV itself is not cytopathic, liver damage in chronic hepatitis C is commonly attributed to immune-mediated mechanisms. HCV proteins interact with several pathways in the host's immune response and disrupt pathogen-associated pattern recognition pathways, interfere with cellular immunoregulation via CD81 binding and subvert the activity of NK (natural killer) cells as well as CD4+ and CD8+ T-cells. Finally, HCV-specific T-cells become increasingly unresponsive and apparently disappear, owing to several possible mechanisms, such as escape mutations in critical viral epitopes, lack of sufficient help, clonal anergy or expansion of regulatory T-cells. The role of neutralizing antibodies remains uncertain, although it is still possible that humoral immunity contributes to bystander damage of virally coated cells via

antibody-dependent cellular cytotoxicity. Cytotoxic lymphocytes kill HCV-infected cells via the perforin/granzyme pathway, but also release Fas ligand and inflammatory cytokines such as IFNγ (interferon γ). Release of soluble effector molecules helps to control HCV infection, but may also destroy uninfected liver cells and can attract further lymphocytes without HCV specificity to invade the liver. Bystander damage of these non-specific inflammatory cells will expand the tissue damage triggered by HCV infection and ultimately activate fibrogenesis.[26, 30]

5.Hepatocellular carcinoma:

A recent re-analysis of the worldwide global burden of cancer places liver as the 5th most prevalent target organ in terms of the estimated new cases in men, and 7th in women. Among many potential etiological factors that have been causally linked to human cancers, including HCC, infectious agents represent an important sub-group of agents that have been classified as ‘‘carcinogenic to humans’’. Evidence exists to suggest that HCV may be both directly and indirectly involved in the development and progression of HCC. The evidence for the direct carcinogenic action of HCV is less prominent than that for other carcinogenic viruses (e.g. papillomaviruses, herpes viruses, Epstein-Barr virus) which integrate into cellular DNA and/or impair normal controls of proliferation and cell death.[2, 11, 31]

HCV is a positive-strand RNA virus that replicates outside of the nucleus and does not have any potential to integrate its genetic information into the host cell’s genome. HCV, however, has been found to be able to change a number of normal molecular pathways that control cell cycle. Most attention has been devoted to the interaction of various HCV non-structural proteins with cellular proteins that control proliferation. For example NS5B has been shown to be bound in the cytoplasm to the retinoblastoma (Rb) protein, the mechanism that is considered to be essential in overcoming infection-induced blocks to cell proliferation. HCV proteins (core, NS3 and NS5A) have also been suggested to disrupt the function of the tumor suppressor p53, which may be a synergistic effect to the loss of Rb. It was also revealed that the viral protein NS4B activates the expression of several members of the PKC superfamily, stimulates the ERK/JNK signaling cascades, and represses SOCS3 expression, resulting in the activation of STAT3 by enhancing its phosphorylation. Activated STAT3 then stimulates MMP-2 and Bcl-2 expression, thereby resulting in deregulation of cell transformation and apoptosis.[32, 33]

The indirect mechanisms of carcinogenesis in the HCV-infected liver are thought to result from the loss of virus-bearing hepatocytes which may lead to the increased rates of proliferation. In addition, chronic inflammation and oxidative stress, coupled with increased proliferation, may result in accumulation of mutations and oncogenic transformation of hepatocytes. The indirect mechanisms of carcinogenesis also seem likely as a relatively small percentage of hepatocytes are HCV-infected in chronic carriers of the virus.[30, 34]

6.Extrahepatic Manifestations

Chronic HCV infection has been associated with numerous extrahepatic manifestations. These manifestations can involve multiple organ systems, including renal, dermatologic, hematologic, and rheumatologic systems. Approximately 1%-2% of HCV-infected individuals will develop extrahepatic manifestations. The most common extrahepatic condition is mixed

cryoglobulinemia. Other frequent extrahepatic manifestations found in patient with chronic HCV infection are membranoproliferative glomerulonephritis, porphyria cutaneous tarda, lichen planus, and vitiligo. There is also some data that suggests an association between chronic HCV infection and non-Hodgkin's and Hodgkin's lymphoma, autoimmune thyroditis, Sjogren's syndrome, and seronegative arthritis. It is unclear if these associated diseases are caused directly from HCV infection or from the underlying immune stimulation caused by chronic infection.[2, 35]

Hepatitis C Global Prevalence:

HCV has been shown to have a worldwide distribution, occurring among persons of all ages, genders, races and regions of the world. The socio-economic burden of HCV has not yet been defined in most countries. Where the epidemiology of hepatitis C has been studied, the consequences of chronic hepatitis C, HCC and end-stage liver cirrhosis have been shown to increasingly impact on national health systems. New infections still occur, because of the continued use of unscreened or inappropriately screened blood transfusions and blood products, the failure to sterilize medical equipment adequately, and the increase in intravenous drug use in previously unaffected areas. Global, regional and national monitoring will be necessary to evaluate results and address shortcomings (Figure 6, Table 4).[24, 36]

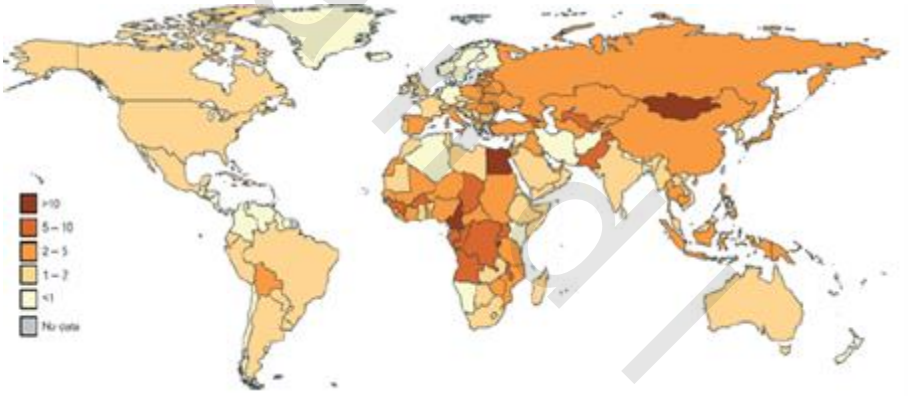

Figure 6: Hepatitis C global prevalence

Table 4: Hepatitis C regional prevalence

Region	Anti-HCV (%)	No. HCV-infected
Africa	3.2	28 100 000
Americas	1.5	14 000 000
Asia	2.1	83 000 000
Australia and Oceania	1.2	400 000
Europe	2.3	17 500 000
Middle East	4.7	16 000 000
Total	2.35	159 000 000

In addition to anti-HCV-based prevalence studies, longitudinal genotype observation adds a further tool for monitoring epidemiological trends. Measurement of the spatial introduction of new genotypes in a population, and the rate of sequence evolution or natural recombinations in viruses introduced at a given time in a cohort, provides the possibility of evaluating the history of the past geographical spread of HCV through different populations, shedding light on the demographic, social and biological factors that are at the basis of ancient and current unrecognized routes of transmission. Genotypes 1–3 have a worldwide distribution. Genotypes 4 and 5 are found principally in Africa, and genotype 6 is distributed in Asia. Endemic areas for specific genotypes are found in West Africa (types 1 and 2), West Central Africa (type 4), and the Indian subcontinent (type 3), Central Africa (type 4) and Southeast Asia (type 6). An endemic area for genotype 5 has not been found, except for a local county in central France (Figure 7).[25, 37, 38]

Figure 7: Global HCV distribution according to genotypes

HCV in Egypt:

Egypt has a very high prevalence of HCV, reaching as much as 14.7%, and the country suffers high morbidity and mortality from chronic liver disease, cirrhosis and HCC. Geographically, the desert areas of Egypt have the lowest rates of anti-HCV positivity; rural areas tend to have higher rates than cities; and rates in the Nile Delta (Lower Egypt) are higher than in the Nile Valley (Middle Egypt and Upper Egypt) (Figure 8). The strong homogeneity of HCV subtypes found in Egypt (mostly 4a) suggests an epidemic spread of HCV. The risk factor(s) originally responsible for the establishment of HCV in the general population may not necessarily be the same as those responsible for transmitting the virus today. The prime candidate to explain the high prevalence of HCV in Egypt is the past practice of parenteral therapy for schistosomiasis with tartar emetic (potassium antimony tartrate), and the data suggest that Egypt's mass campaigns do indeed represent the world's largest example of iatrogenic transmission of a blood-borne pathogen; the large reservoir of chronic HCV infection established in the course of these campaigns remains be largely responsible for the continuing endemic transmission of HCV today . [39-41]

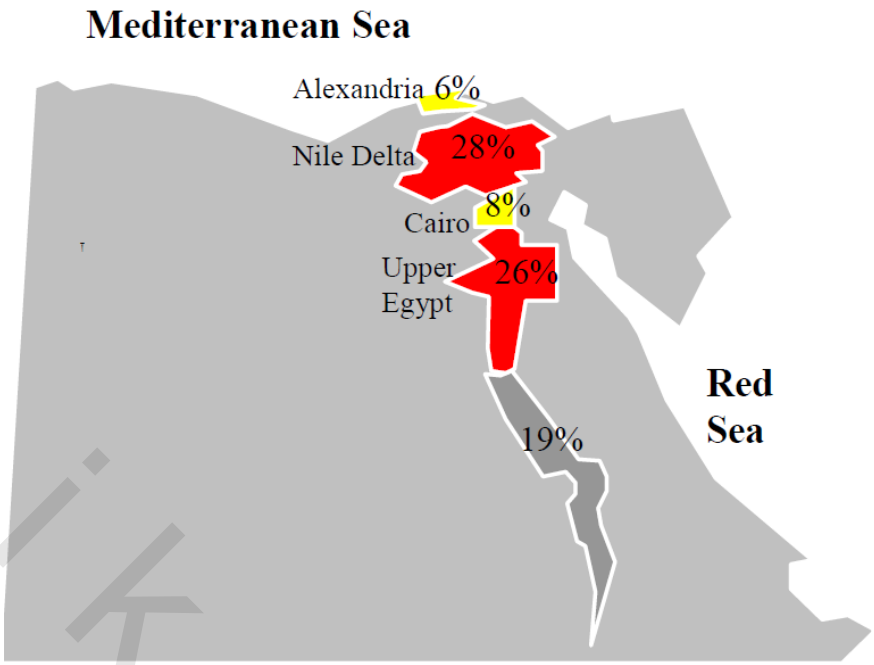

Figure 8: HCV genotype distribution in Egypt.

Egyptian Demographic Health Survey 2008:

Viral hepatitis is the most significant public health problem facing Egypt today. HCV prevalence rates in the general population are estimated at between 10% and 15% in rural areas, with some age groups suffering from prevalence rates of up to 50%. Incidence rates are estimated at 2-6 per 1,000 per year, a level that will maintain prevalence rates of 5-15% for the foreseeable future. The virus continues to be transmitted in medical and paramedical settings, as well as within communities and families. Approximately 8 million Egyptians carry antibodies for HCV. Though not all persons infected with HCV proceed to develop cirrhosis of the liver or other life-threatening sequelae, the medical and economic burden incurred by those who do is significant. Liver disease is a top cause of mortality in Egypt, and mathematical models predict an upsurge in cases of liver cirrhosis and liver cancer in the years to come unless approached properly.[39, 40, 42, 43]

Epidemiological and molecular studies in Egypt relate the origin of the HCV-4 epidemic to the mass antischistosomal campaign, which was administered parenterally, and only stopped in the mid-1960s. However, the incidence of HCV remains high even after the treatment campaign was stopped and new infections continue to occur in young individuals who did not receive parenteral antischistosomal therapy. Blood transfusion was a major route for HCV-4 transmission before obligatory HCV screening in blood banks in 1994. Currently, the major route of transmission appears to be health-related procedures with inadequately sterilized instruments and supplies. Occupational transmission among healthcare workers through needle sticks and injuries from sharp objects contributes to new HCV cases.[39, 44, 45]

As for studies concerning intra-familial transmission in Egypt, previous analyses showed that no risk factor could explain the infections among children aged 5-15 years. Another unusual feature of the epidemic in Egypt was the important clustering of infections at the household level, suggesting either, common at-risk behaviours, intra-familial transmission, or genetic susceptibility to infection or disease. Phylogenetic analysis showed greater HCV strain similarity between family members than between unrelated subjects indicating that correlations can be explained in part by familial sources of virus transmission. In addition, refined dissection of correlations between first degree relatives supported the role of host genes predisposing to HCV infection. Studies found evidence for a dominant major gene predisposing to HCV infection. The predisposing allele frequency was 0.013, indicating that 2.6% of the subjects, in particular those younger than 20 years old, were predisposed to HCV infection.[46-49]

HCV transmission patterns

The risk factors most frequently cited as accounting for the bulk of HCV transmission worldwide are blood transfusions from unscreened donors, injection drug use, unsafe therapeutic injections, and other health-care related procedures. Most developed countries have accumulated evidence that the predominant source of new HCV infections within their borders over the past few decades is injection drug use. In the developing world, unsafe therapeutic injections and transfusions are likely to be the major modes of transmission, especially in countries where age-specific seroprevalence rates suggest ongoing increased risk of HCV infection. In developed countries with high seroprevalence in older age groups, unsafe therapeutic injections probably had a substantial role in HCV transmission 30–50 years ago, and may persist as an important cause of transmission in isolated, hyperendemic areas.[50-52]

Blood transfusion

Blood transfusion is a highly effective means of transmitting HCV infection. In most of the developed world, numerous measures over the past four decades have resulted in progressive reductions in the risk of transfusion-transmitted HCV infection. These measures include adoption of an all-volunteer donor system, screening of blood donations with surrogate laboratory tests for liver disease, screening of potential donors based upon answers to questions related to HIV risk factors, anti-HCV testing, and HCV nucleic acid testing.[53, 54]

Unsafe therapeutic injections

In the developed world, the relative contribution of health-care-related transmission of HCV infection to overall HCV infection transmission is difficult to quantify, but likely small, despite numerous recent outbreaks stemming from lapses in aseptic techniques and infection control practices. However, in many developing countries, supplies of sterile syringes may be inadequate or non-existent, non-professionals often give injections outside the medical setting, and injections are often given to deliver medications that could otherwise be delivered by the oral route. In this environment people may receive multiple contaminated injections over the course of a lifetime, incurring a substantial cumulative risk of HCV infection.[55, 56]

Injection drug use

Injection drug use is the primary mode of transmission for HCV infection in the developed world. In countries such as the USA and Australia, where the highest seroprevalence is among middle-aged people, injection drug use has been the dominant mode of transmission for more than 30 years.[52, 57]

Other sources of HCV transmission

Transmission of HCV infection through occupational, perinatal, and sexual exposures occurs with much less efficiency compared with transmission through large or repeated percutaneous exposures. Thus, occupational, perinatal, and sexual transmission are unlikely to be major sources of new HCV infections, regardless of the population or geographic area. Occupational transmission of HCV infection is largely confined to health-care workers who have sustained a contaminated needlestick injury, and observed attack rates under these circumstances are as low as 0.3%. Acquisition of HCV infection through perinatal transmission is estimated to occur in 2.7–8.4% of infants born to HCV infected mothers, and a higher proportion of infants born to HIV/HCV coinfected mothers. Sex with an infected partner and with multiple partners have been identified as risk factors for HCV transmission, but sexual transmission of HCV is far less efficient than that of other sexually transmitted viruses.[51, 53, 58]

Because of the wide variety of human activities that involve the potential for percutaneous exposure to blood or blood-derived body fluids, there are many modes of transmission besides those with clearly demonstrated epidemiological associations with infection. These modes of transmission include cosmetic procedures and religious or cultural practices such as tattooing, body-piercing, commercial barbering, ritual scarification, circumcision, acupuncture, and cupping.[51, 53, 58]

Hepatitis C Virus Genotypes

5' UTR has a complicated secondary structure comprised of at least seven stem-loops important for ribosome entry and presumably, for viral RNA replication. The translation of HCV RNA begins at the internal ribosome entry site (IRES) and at the 40S ribosomal subunit in the absence of external factors, which makes the HCV translation efficient. Thus, the secondary structure of IRES plays an important role in HCV replication, and single nucleotide changes within this region could alter viral replication characteristics.

HCV replication via RNA-dependent RNA polymerase is very error-prone and generates mutations at an estimated rate of 10^5 mutations per nucleotide per replication. This high mutation rate is the ultimate source of the virus's genetic diversity. HCV circulates as a heterogeneous population of genetically different but closely related genomes known as the quasispecies.

As only 30-35% of nucleotides actually differ between genotypes, there is obviously considerable heterogeneity in evolutionary rates among nucleotide sites in the genome. This heterogeneity is the result of variable evolutionary constraints. The 5'-UTR contains extensive secondary RNA structure and is correspondingly the slowest evolving genomic region. The next slowest region is the C (Core) gene, which evolves three times faster than the 5'- UTR. The envelope genes E1 and E2 constitute the most diverse genome region and evolve about nine times faster than the 5'-UTR, probably as a result of their presumed role in evading the host immune response.[59-61]

Genomic Heterogeneity and Classification Systems

Shortly after its discovery in 1989, it became clear that HCV had substantial nucleotide sequence diversity, with only 66 to 80% overall sequence similarity among strains belonging to different genotypes or subtypes. HCV isolates show four levels of genomic variations: types, subtypes, isolates, and quasispecies. The overall sequence similarities over complete genomic sequences are at least 91% within quasispecies, approximately 79% (range, 77 to 80%) between subtypes, and about 68% (range, 66 to 69%) between different types.[59-61]

This quasispecies is composed of a group of heterogeneous RNA sequences centered around a dominant nucleotide sequence that changes, throughout the course of the infection, under the selective pressure of the host immune system. More than one genotype can be found in the circulations of some HCV-infected patients, particularly in individuals who have received multiple transfusions and intravenous drug users. These are referred to as mixed-genotype infections. The lack of a routinely available cell culture system and an easily available animal model has rendered classification of HCV, based on its phenotype according to its disease pattern or cytopathology, and serotype based on a panel of cross-neutralization antibodies, impossible. [23]

Phylogenetic analysis may aid in the separation of sequences into distinct types. So far six major genotypes (HCV-1 to HCV-6) have been described, each containing multiple subtypes (e.g., 1a, 1b, etc.). More than 50 subtypes have been reported to date and are normally identified on the basis of partial gene sequences from E1 and NS5B. The isolates formerly published as genotypes 7 to 11 are now considered subtypes within genotypes 3 (subtype 10) and 6 (subtypes 7, 8, 9, and 11. In general, sequence characteristics of a particular subtype are found throughout the HCV genome. Thus, the HCV genotype has been determined primarily based on analysis of partial genome sequences. The most extensive database exists for the 5'-UTR, core, E1, and NS5B . Whereas the 5'-UTR is highly conserved and therefore preferred for diagnosis, the core, the envelope, and the NS5B regions are less conserved and therefore highly discriminative and preferred for subtyping. Although the 5'-UTR contains characteristic sequence motifs of some genotypes, analysis of this region may not accurately predict all genotypes or subtypes.[25, 62]

Clinical Relevance of HCV Genotypes

Genotype, viral load, and liver histology are important parameters used in selecting an antiviral therapy with the greatest chance of success. Genotyping and subtyping of HCV is relevant to the epidemiology of HCV, vaccine development, clinical management, and assessment of the risk benefit ratio of therapeutic measures against chronic HCV infection. It has been postulated that differences in nucleotide sequence could result in differential activity of HCV proteins that could alter the rate of HCV replication, sensitivity to the antiviral activity of interferon, or pathogenicity of the virus. Genotype 1 in particular cannot be treated efficiently with IFN-alfa, while genotypes 2 and 3 respond favorably. The causes of variation in treatment response are not well understood. Studies indicated that the outcome of interferon therapy was correlated with genetic variability in a portion of the NS5A gene (the interferon sensitivity determining region, ISDR). [63]

HCV Genotyping Methods

Molecular Genotyping

Because differences in geographical distribution, disease outcome, and response to therapy among HCV genotypes have been suggested, reliable methods for determining the HCV genotype may become an important clinical test. The HCV genotype can be determined by nucleotide sequencing of a specific PCR-amplified portion of the HCV genome obtained from the patient, followed by composition of a phylogenetic tree, which is presently the 'gold standard' for the detection and identification of the various HCV genotypes and subtypes.

Investigators of HCV genotyping have used sequence analysis of HCV NS5, core, E1, and 5'-UTRs. This approach, however, is regarded as impractical for routine clinical laboratory settings. More convenient methods focus on the amplification of defined regions of the HCV genome by reverse transcription (RT)-PCR followed by digestion with restriction enzymes and restriction fragment length polymorphism analysis (RFLP), amplification with genotype-specific primers, hybridization of genotype-specific probes with the amplified products, heteroduplex mobility assay, melting curve analysis with fluorescence resonance energy transfer probe and DNA enzyme assays. The reference method for HCV genotype determination is direct sequencing of the NS5B or E1 regions of HCV genome by means of “in-house” techniques,

followed by sequence alignment with prototype sequences and phylogenetic analysis. These techniques must be used in molecular epidemiology studies, where exact subtyping is needed. In clinical practice, HCV genotype can be determined by various commercial kits, using direct sequence analysis of the 5' noncoding region (Trugene® 5'NC HCV Genotyping Kit, Bayer HealthCare, Diagnostics Division, Tarrytown, New York) or reverse hybridization analysis using genotypespecific probes located in the 5' noncoding region (commercialized as INNO-LiPA HCV II, Innogenetics, Ghent, Belgium, or Versant® HCV Genotyping Assay, Bayer HealthCare). Mistyping is rare with these techniques, but mis-subtyping may occur in 10 to 25% of cases, related to the studied region (5' noncoding region) rather than the technique used. These errors have no clinical consequences, because only the type is used for therapeutic decision-making. An assay based on direct sequencing of the NS5B region is currently in development (Trugene® NS5B HCV Genotyping Kit, Bayer HealthCare).[64, 65]

Serologic Genotyping

Investigators identified genotype specific antibodies that could be used as indirect markers for the HCV genotype. Serological typing uses enzyme immunoassays to detect the antigenic properties of several specific epitopes encoded by the NS-4 or the core regions of the HCV genome. Serologic genotyping has several advantages that make it suitable for large epidemiologic studies. These advantages include the low risk of contamination and the simplicity of the assay. However, serologic typing seems to lack specificity and sensitivity, which limits its usefulness.[59, 60]

Hepatitis C Virus Serologic and Virologic Tests and Clinical Diagnosis

The use of serological and virological tests has become essential in the management of hepatitis C virus infection in order to diagnose infection, guide treatment decisions and assess the virological response to antiviral therapy. Virological tools include serological assays for anti-HCV antibody detection and serological determination of the HCV genotype, and molecular assays that detect and quantify HCV RNA and determine the HCV genotype. Anti-HCV antibody testing and HCV RNA testing are used to diagnose acute and chronic hepatitis C.[12, 66]

Molecular assays for hepatitis C

This can be divided into three general categories: 1) tests that detect the presence or absence of the HCV RNA genome in patient plasma or serum (eg, qualitative HCV RNA tests); 2) tests that assess the quantity of HCV RNA in the blood, otherwise known as the viral load (eg, quantitative HCV RNA tests); and 3) tests that determine the genetic nature of HCV (eg, HCV genotype tests). Patients who test positive for HCV RNA in the blood are known to be actively infected by HCV and are at increased risk for developing significant liver disease.[12, 67]

Detection and quantification of HCV RNA

Qualitative, non-quantitative HCV RNA detection

Qualitative detection assays are based on the principle of target amplification using either "classic" polymerase chain reaction (PCR), "real-time" PCR or TMA (transcription-mediated amplification). HCV RNA is extracted and reverse transcribed into a double stranded complementary DNA (cDNA), which is subsequently processed into a cyclic enzymatic reaction leading to the generation of a large number of detectable copies. Double-stranded DNA copies of HCV genome are synthesized in PCR-based assays, whereas single-stranded RNA copies are generated in TMA. Detection of amplified products is achieved by hybridizing the produced amplicons onto specific probes after the reaction in "classic" PCR or TMA techniques.[12, 68, 69]

Qualitative detection assays must detect 50 HCV RNA IU/ml or less, and have equal sensitivity for the detection of all HCV genotypes. The lower limit of detection of the qualitative, non quantitative reverse transcriptase PCR-based assay Amplicor® HCV v2.0, or of its semi-automated version Cobas® Amplicor® HCV v2.0 (Roche Molecular Systems, Pleasanton, California) is 50 IU/ml, whereas that of the TMA-based assay Versant® HCV RNA Qualitative Assay (Bayer HealthCare) is 10 IU/ml . Real-time PCR assays, which are also able to quantify HCV RNA, have lower limits of detection of the order of 5-30 IU/ml when they are used as purely qualitative, non-quantitative assays.[66, 70, 71]

HCV RNA quantification

HCV RNA can be quantified by means of target amplification techniques (competitive PCR or real-time PCR) or signal amplification techniques (branched DNA (bDNA) assay). In "real-time" PCR, each round of amplification leads to the emission of a fluorescent signal and the number of signals per cycle is proportional to the amount of HCV RNA in the starting sample. Five standardized assays are commercially available. Two of them are based on competitive PCR: Amplicor HCV Monitor® v2.0 and its semi-automated version Cobas® Amplicor HCV Monitor® v2.0 (Roche Molecular Systems), and LCx® HCV RNA Quantitative Assay (Abbott Diagnostic); one is based on bDNA technology, Versant® HCV RNA 3.0 Assay (Bayer Healthcare); and two are based on real-time PCR amplification, Cobas® TaqMan HCV Test, which can be coupled with automated extraction in Cobas Ampliprep® (Roche Molecular Systems), and Abbott RealTime™ HCV assay (Abbott Diagnostics), which uses the Abbott m2000 system and can also be coupled with an automated extraction procedure. [14, 15, 67, 72]

Serological assays for anti-HCV:

Serological assays have been subdivided into screening tests for anti-HCV, such as the enzyme immunoassay (EIA), and supplemental tests such as the recombinant immunoblot assay (RIBA). Three generations of anti-HCV tests have been developed, and each generation has resulted in an improvement in the sensitivity of detecting anti HCV. Supplemental anti-HCV tests are designed to resolve false-positive testing by EIA, and are appropriately used in low-prevalence settings in which false-positive anti-HCV tests remain a problem.[73]

Screening tests

The main screening assay for detecting anti-HCV is the enzyme immunoassay (EIA). The EIA has many advantages in the diagnostic setting, including ease of use, low variability, ease of automation, and relatively low expense. The first-generation anti-HCV test (EIA- I) contained a single HCV recombinant antigen derived from the nonstructural (NS 4) gene, designated c-l00-3. Although development of this test represented a dramatic breakthrough in terms of diagnosing HCV infection and reducing HCV transmission via blood transfusion, EIA-I lacked optimal sensitivity and specificity and was subsequently replaced in 1992. The EIA-2 test contains HCV antigens from the core and NS3 genes in addition to the NS4 antigen, and thus represents a multi-antigen EIA. Introduction of the new antigens led to a substantial improvement in sensitivity and a slight increase in specificity relative to the EIA- I. The use of core and NS3 antigens in the EIA-2 test shortened the average "window period" for HCV seroconversion by 4-10 weeks relative to the EIA-I test. A third-generation antiHCV test (EIA-3), which contains reconfigured core and NS3 antigens plus an additional HCV antigen (NS5) not present in the EIA-2, has been approved for screening blood products. While preliminary studies suggest an incremental improvement in sensitivity in blood donors, immunosuppressed populations, and liver clinic populations, the specificity of the EIA-3 test has not been adequately defined in the routine diagnostic setting, and utility of the NS5 antigen in this assay has been controversial.[12, 74, 75]

A progressive improvement in sensitivity of detection of anti-HCV has been accomplished by the three generations of EIA screening assays. However, testing in high-prevalence populations has indicated that not all patients with active HCV infection (e.g., HCV RNA positive) are identified with the EIA screening tests. Studies suggest the envelope 2 (E2) antigen may be a good candidate for subsequent versions of the EIA test. Although false-positive EIA testing remains a problem in low-prevalence populations, the accuracy of the EIA-2 test is very good in high-prevalence populations, and therefore, supplemental anti-HCV tests may not be necessary in high-risk patients with a positive anti-HCV screen.[66, 73]

Combination antigen-antibody assays were introduced, where two markers of the same infection could be detected simultaneously. These assays came to be known as "fourth generation" or "antigen-antibody combo" tests and appeared more suitable in a blood bank setting where large numbers of donor samples need to be screened in the shortest possible time. The combined antigen-antibody assays are usually sandwich ELISAs where the solid phase and second phase comprise both HCV derived antigens and antibodies against HCV. Combined assays resulted in suboptimal detection of HCV antigen when compared to antigen only assays. Despite this, combination assays are a definite improvement over antibody only assays, as they detect the infection earlier and are capable of detecting immunosilent carriers who are viremic without detectable antibodies in their plasma.[76]

Supplemental tests:

Supplemental tests for anti-HCV were developed to help resolve false-positive EIA test results. The prototype supplemental test in the United States is the FDA-licensed second-generation recombinant immunoblot assay (RIBA-2), which contains the same HCV antigens as EIA-2 in an immunoblot format. A third-generation supplemental test (RIBA-3) has been introduced in Europe, which appears to be more specific than the RIBA-2 test based on a better correlation with RNA PCR results and a reduced number of RIBA-indeterminate results.[27, 69, 77]

Diagnosis of HCV infection:

Acute hepatitis C

Patients with a suspicion of acute hepatitis C should be tested for both anti-HCV antibodies by EIA and HCV RNA with a sensitive technique, i.e. an HCV RNA assay with a lower limit of detection of 50 IU/ml or less. Four marker profiles can be observed according to the presence or absence of either marker. The presence of HCV RNA in the absence of anti-HCV antibodies is strongly indicative of acute HCV infection, which will be confirmed by seroconversion (i.e. the appearance of anti- HCV antibodies) a few days to weeks later. Acutely infected patients can also have both HCV RNA and anti- HCV antibodies at the time of diagnosis. It is difficult, in this case, to distinguish acute hepatitis C from an acute exacerbation of chronic hepatitis C or an acute hepatitis of another cause in a patient with chronic hepatitis C. Acute hepatitis C is very unlikely if both anti-HCV antibodies and HCV RNA are absent. It is also unlikely if anti-HCV antibodies are present without HCV RNA. These patients should however be retested after a few weeks because HCV RNA can be temporarily undetectable, due to transient, partial control of viral replication by the immune response before replication escapes

and chronic infection establishes. Apart from such cases, the presence of anti-HCV antibodies in the absence of HCV RNA is generally seen in patients who have recovered from a past HCV infection. Nevertheless, this pattern cannot be differentiated from a false positive EIA result, the exact prevalence of which is unknown.[27, 66]

Chronic hepatitis C

In patients with clinical or biological signs of chronic liver disease, chronic hepatitis C is certain when both anti- HCV antibodies and HCV RNA (sought for with a sensitive technique, detecting 50 IU/ml or less) are present. Detectable HCV replication in the absence of anti-HCV antibodies is exceptional with the current third generation EIAs, almost exclusively observed in profoundly immunodepressed patients, hemodialysis patients or agammaglobulinemic subjects. In patients who have no indication for therapy or have a contra-indication to the use of antiviral drugs, virological tests have no prognostic value.

Indeed, neither anti-HCV antibodies nor the HCV RNA load correlate with the severity of liver inflammation or fibrosis nor with their progression. Thus, they cannot be used to predict the natural course of infection or the onset of extrahepatic manifestations. In untreated patients, the severity of liver inflammation and fibrosis must be evaluated every three to five years by means of a liver biopsy or non-invasive serological or ultrasound-based testing.[37, 67, 69, 78]

Therapy for Hepatitis C

Goals of therapy:

The ultimate goals of antiviral therapy are to eliminate HCV, prevent transmission, improve or normalize the liver tests and histology (microscopic appearance), prevent progression to cirrhosis and liver cancer, prolong survival, and improve the quality of life.[1, 50, 52, 79-81]

Indications for Therapy

Therapy for hepatitis C is clearly indicated in patients 18 to 60 years of age who have persistently abnormal alanine aminotransferase levels, HCV RNA in serum, and evidence on liver biopsy of chronic hepatitis with either fibrosis or moderate degrees of inflammatory activity.[50]

Optimal Therapeutic Regimen

The National Institute of Health (NIH) Consensus Panel stated that the optimal therapeutic regimen for hepatitis C was interferon- alpha given subcutaneously in a dose of 3 million U three times weekly for 12 months with assessment of aminotransferase levels and HCV RNA at 12 weeks (early virological response) to allow early discontinuation in patients who do not respond. Two years later (1997), these recommendations required modification in response to results of studies on combination therapy with interferon-alpha and ribavirin.

Ribavirin is an oral nucleoside analogue with a broad spectrum of activity against both RNA and DNA viruses. When used alone as therapy for hepatitis C, ribavirin decreases aminotransferase levels and improves hepatic histologic findings in 30% to 50% of patients. However, HCV RNA levels do not decrease, and relapses occur in almost all patients soon after treatment is stopped.

Despite the impressive results obtained with combination therapy, the real issue is whether patients in whom sustained virologic response (RNA level 48 weeks after continuation of therapy) is achieved are likely to have relapse months or years later. Long-term follow-up after treatment with interferon alone indicates that most patients who fulfill the criteria for a sustained virologic response remain negative for HCV RNA, have normal serum aminotransferase levels, and have no symptoms of liver disease 5 to 12 years after treatment.

Some patients treated successfully with combination therapy still have detectable virus after 12 weeks of treatment but go on to have a sustained response. Therefore, patients on combination therapy should have hepatitis C virus RNA measured at 24 weeks of therapy. In those who are still positive for the virus at that time, consideration is given to stopping treatment, since the chance of sustained response is small.[1, 27, 82, 83]

Limitations of the Standard-of-Care Treatment (Combined interferon alpha plus ribavirin):[80, 83, 84]

- 1. Non specific action: Interferon activates immune system and inhibits viral replication. Ribavirin inibits viral replication.
- 2. Contraindications: Interferon: autoimmune diseases, uncontrolled depression and mental illness, decompensated liver disease or decompensated cardiac or pulmonary disease. Ribavirin: Pregnant patients or those with advanced renal disease
- 3. Side effects: Interferon: flu-like, headache, myalgia, depression, thrombocytpenia, neutropenia . Ribavirin: hemolytic anemia, teratogenic
- 4. Failing therapy due to relapse, non or null response.

Factors That Predict Response to Therapy:

The host factors of young age, female sex, lesser degrees of fibrosis on liver biopsy, lower ALT levels,and lower body mass index, correlated with a greater likelihood of a sustained response. Also the host genetic factors play a role, where independent genome-wide association studies (GWAS) revealed that some SNPs around IL28B on chromosome 19 which codes for interleukin 28B (IFN- λ3), is associated with both treatment induced and spontaneous HCV clearance. Even more significant were the viral features of genotype and HCV RNA level. The sustained response rates to standard of care among patients with genotypes 2 and 3 was twice as high (80%) as that among patients with genotype 1 (40-50%), whereas response to genotype 4 was intermediate (50-60%). [50, 85-88]

How are relapses and non responders treated?

The optimal treatment for non responders and relapsers is not well established. A minority of non responders (6% to 12%) will respond to a second course of pegylated interferon and ribavirin. Patients initially treated with older non pegylated interferon can be considered for the therapy with either pegylated interferon or pegylated interferon plus ribavirin therapy. Newer preparations of interferon and protease inhibitors are being studied and show promise in persons who did not respond to combination therapy.[26, 83, 84]

Liver transplantation for hepatitis C infection

Finally, if the liver is severely damaged, a liver transplant may be recommended. End-stage liver disease (cirrhosis) due to chronic Hepatitis C viral infection is the number one reason for liver transplantation in the United States. During the transplant procedure, the diseased liver will be replaced with a healthy liver from an organ donor or from a live donor who donates a portion of their healthy liver. However, contrary to popular misconception, a liver transplant is not a cure for Hepatitis C. Unfortunately, the Hepatitis C virus recurs in the new liver in almost all cases, with fibrosis (scarring of the liver) or cirrhosis occurring in 10% to 30% of patients in as little as 5 years after the transplant.

Treatment for recurrent hepatitis after transplantation is not a simple issue. Most transplant centers delay therapy until recurrent disease is confirmed. Treatment of recurrent hepatitis is complicated with interferon, an important drug for treatment, and an immune modulator that may promote rejection of the transplanted liver. Furthermore, interferon and ribavirin may not be well tolerated by patients who just underwent transplantation and are taking many different kinds of medications.[79, 89]

Table 5: Definitions of virologic response to HCV treatment

Response	Definition
Rapid virologic response	Undetectable HCV RNA at treatment wk 4
Early virologic response	≥2 log reduction of HCV RNA at treatment wk 12 compared with baseline level by quantitative HCV-RNA assay
Complete early virologic response	Undetectable HCV RNA at wk 12 in absence of rapid virologic response
Partial early virologic response	≥2 log reduction of HCV RNA, but without undetectable HCV RNA at treatment wk 12 in absence of rapid virologic response
End-of-treatment virologic response	Undetectable HCV RNA at end of treatment
Sustained virologic response	Undetectable HCV RNA 24 wk after treatment completion

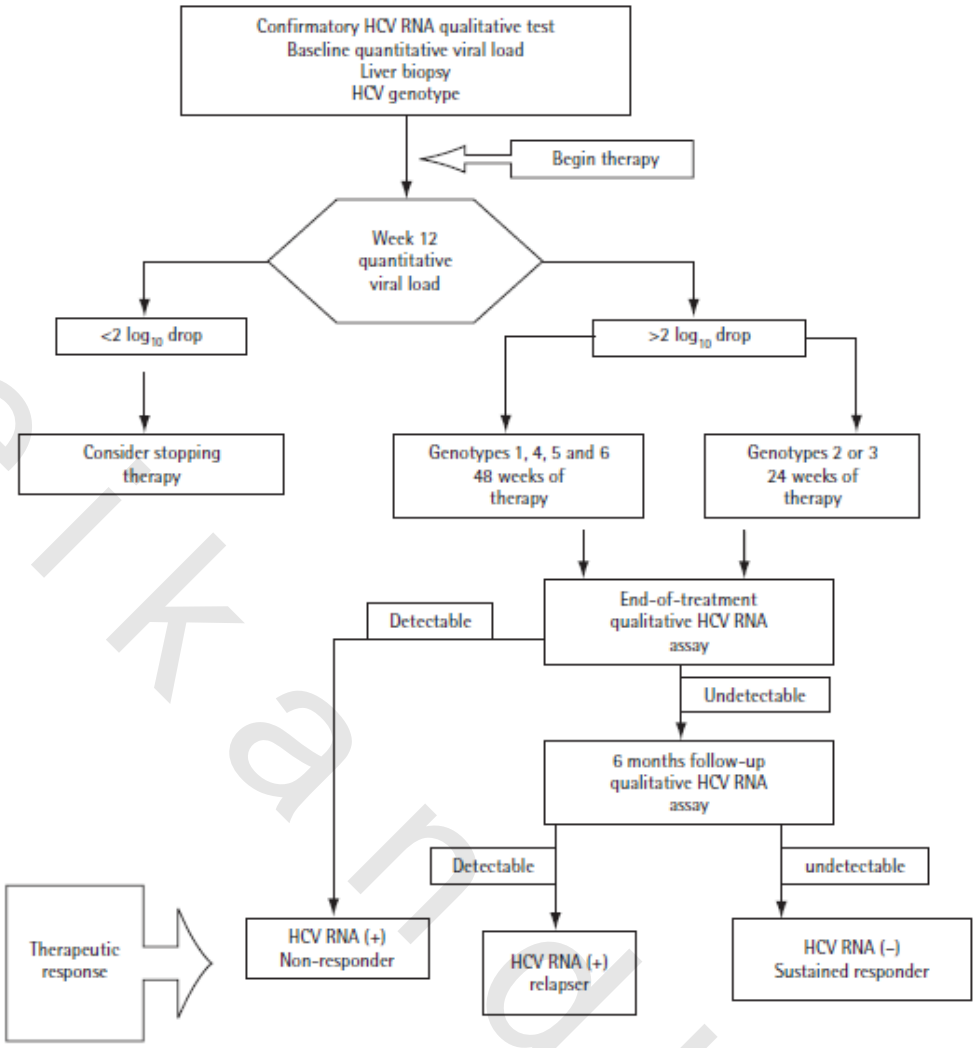

Figure 9: Algorithm of management of HCV infection

Egyptian National Control Strategy for HCV:

Since 2006, Egypt has made great progress in the management of viral hepatitis in establishing a National Committee for the Control of Viral Hepatitis with 23 affiliated viral hepatitis treatment units distributed all over the country as a part of Viral Hepatitis National Treatment Program launched in 2008. Between 2006 and 2012, more than 220 000 HCV patients were treated in National Treatment Centers and HIO centers. The estimated cost of this program to the Egyptian government is $80 million annually, which covers 40% of the total costs; remaining 60% is paid by insurance companies (50%) and patients (10%).[87, 90]

Treatment guidelines for HCV (2008 – 2012)

Currently, Egyptians being treated for HCV receive 48 weekly subcutaneous doses of pegylated interferon with twice daily doses of ribavirin, at a total cost of approximately 25,000 LE (€3,000). The drugs are produced by the pharmaceutical companies Roche and Schering.[40, 91, 92]

1) Inclusion and exclusion criteria:

a) Age is from 18 to 60 years. Those <18 years will be managed through clinical trials supervised by pediatricians.

b) Liver biopsy is mandatory.

c) Compensated liver cirrhosis (Child A) will be treated if there are no varices.

d) Mild changes in the liver biopsy >F0 & >A1 with elevated liver enzymes will be treated.

e) Those with normal liver enzymes and Metavir score ≥A2 and ≥F2 will be treated.

f) BMI >35 will not be treated except after weight loss.

g) Treatment is contraindicated for patients with autoimmune diseases, uncontrolled depression and mental illness, decompensated liver disease or decompensated cardiac or pulmonary disease, pregnant patients or those with advanced renal disease.

h) Regulations for the special groups like non responders, chronic renal failure, thalassemia and liver transplantation will be managed by experienced doctors until separate guidelines have been created for them.

2) Stoppage rules:

Treatment will be discontinued for these who are still PCR HCV positive after 12 weeks or with no 2 log decrease. At 24 weeks treatment will be stopped for all patients with viremia. Those with negative PCR at 24 & 36 weeks will continue for the full 48 weeks.

3) Blood samples will be collected and preserved at -80Cº at 0, 1, 3, 6, 12 and 18 months from treatment debut, for the purposes of research.

4) Suggested research topics will be raised by the members of the Advisory Committee and conducted under their supervision.

Innovative agents in Clinical Development:

For the development of new, specific anti-HCV drugs, an understanding of the HCV replicative cycle, in particular the genomic organization and polyprotein processing, is essential.

This has resulted in the development of several agents that target specific stages of the life cycle, the so-called specifically targeted antiviral therapy for HCV (STAT-C) drugs (Directly acting anti viral drugs (DAA).[91, 93, 94]

Potential processes for viral inhibition:

- Virus entry into the host cell
- Proteolytic processing
- RNA replication
- Assembly and release

Among the most promising new agents in development are the protease and polymerase inhibitors. [95-98]

I- Post translational modification (*Protease inhibitors):*

The non-structural protein NS3 possesses a protease domain that is responsible for polyprotein processing and is a potential target for antiviral intervention. Despite the catalytic site being a shallow and largely hydrophobic groove, making it difficult to target, several compound inhibitors of the NS3 protease have been successfully designed.[70, 95-97]

FDA approved protease inhibitors:

1. Boceprevir (Victrelis)

On May 13, 2011 FDA approved Boceprevir as DAA for CHC. Boceprevir is used for patients who still have compensated liver function, and who either are treatment naive or who have failed such treatment. Boceprevir is approved for use in combination with peginterferon alfa and ribavirin. The daily dose is 12 capsules—four 200 mg capsules every 7 to 9 hours.

2. Telaprevir (Incivek)

On May 23rd 2011, FDA approved INCIVEK (telaprevir), a hepatitis C virus (HCV) protease inhibitor. Combination triple therapy with this drug is indicated for: CHC patients with compensated liver disease, including cirrhosis, treatment-naïve, previously treated with interferon-based treatment and not responded adequately, null responders, partial responders, and relapsers. The recommended dose of Incivek tablets is 750 mg (two 375-mg tablets) taken orally 3 times a day.

3. Simeprevir: (Olysio)

On November 25th 2013, FDA approved a new drug for chronic hepatitis C infection that some experts hope will cut down on side effects from current therapies. It is approved as part of a combination antiviral drug regimen to treat certain classes of adult patients with hepatitis C. These include patients who have cirrhosis or other liver disease but whose liver is compensated, treatment naïve patients, or those whose infection has not improved after prior treatment.

Limitations of First-generation Direct-acting Antiviral Therapy (Telaprevir and Boceprevir) : [63, 95, 99, 100]

- Moderate-to-severe anemia is common (50% boceprevir, 36% telaprevir) , thrombopenia and neutropenia.
- Many still do not achieve SVR (uncertain cost effectiveness)
- Drug–drug interactions limit use with common medications as statins, contraceptives..
- High pill burden makes compliance difficult
- Resistance is still a real threat
- New rashes and anorectal symptoms (hemorrhoids, burning,itching)
- In December 2012, a black box warning was added to telaprevir labeling in light of some rashes resulting in death

II-Inhibition of RNA replication (*Polymerase inhibitors):*

The protein NS5B is cleaved from the HCV polyprotein by the NS3 serine protease, and functions as a RNA-dependent RNA polymerase. It is the key enzyme for synthesis of a complementary minus strand RNA, using the genome as a template, and the subsequent synthesis of genomic plus-strand RNA from this minus-strand RNA template. This enzyme is essential for viral replication and no host equivalent exists, making it an attractive target.[1, 94, 97, 101-103]

Two structurally distinct classes of inhibitors with different modes of action have been reported: 1)***Nucleoside analogue*** generally target the polymerase active site in a competitive manner and typically show broad-spectrum activity (Valopicitabine 2007 trials stopped because of GIT side effects).2)***Non-nucleoside inhibitors*** have much greater specificity and act either by direct interference with the active site or by binding to an allosteric site and preventing the initiation process.

FDA approved polymerase inhibitor : Sofosbuvir (Sovaldi)

On Decemeber 6th 2013, FDA approved the first polymerase inhibitor as a component of a combination antiviral treatment regimen. Sovaldi is the first drug that has demonstrated safety and efficacy to treat certain types of HCV infection without the need for co-administration of interferon. Efficacy has been established in subjects with HCV genotype 1, 2, 3 or 4 infection, including those with hepatocellular carcinoma and those with HCV/HIV-1 co-infection. The recommended dose of Sovaldi is one 400 mg tablet, taken orally, once daily.

III.Prevention of functional replication complexes: [88]

Ledipasvir-sofosbuvir *(Harvoni)*

On October 10, 2014, the fixed-dose combination ledipasvir-sofosbuvir (*Harvoni*) was approved by the FDA for the treatment of chronic hepatitis C genotype 1 infection in adults. Ledipasvir is a potent inhibitor of HCV NS5A, a viral phosphoprotein that plays an important role in viral replication, assembly, and secretion. The fixed dose combination ledipasvir-sofosbuvir (90 mg/400 mg) is indicated for: Genotype 1 treatment-naïve patients with or without cirrhosis: 12 weeks, genotype 1 treatment-experienced patients without cirrhosis: 12 weeks and genotype 1 treatment-experienced patients with cirrhosis: 24 weeks. A treatment duration of 8 weeks can be considered in treatment-naive patients without cirrhosis who have a baseline HCV RNA level less than 6 million IU/mL.

Harvoni is approved as a combined-pill, an interferon-free regimen for genotype 4 as recommended by the American association for liver diseases and by the European commission.[94, 101]

IV-Inhibition of protein translation: [50, 63, 80, 83, 98, 104]

1- Antisense oligonucleotide:.

Are short synthetic nucleic acids (usually with<25 nucleotides) that bind an RNA target forming RNA–RNA (antisense RNA) or RNA–DNA (antisense DNA) hybrids resulting in inhibition of RNA translation of viral proteins and ⁄ or replication. Several oligonucleotides targeting the 5'-UTR, the most conserved region of the HCV genome, have been reported to inhibit HCV gene expression in vitro. ISIS 14803 is a 20-base antisense oligonucleotide, which is complementary to the HCV translation initiation region within the IRES. ISIS 14803 gave promising results in early phase II clinical trials, but subsequent aminotransferases flares and poor anti-viral efficacy led to discontinuation of further studies.

2-RNA interference:

RNA interference is a method of specific degradation of messenger RNA leading to RNA silencing. BLT-HCV (Benitec), the first clinical candidate to treat HCV infection through RNA interference, consists of three components targeting different HCV sequences, underlining the importance of a multi-targeting approach to prevent resistance development.

3- Ribozymes:

Ribozymes are synthetic nuclease-resistant catalytic RNA molecules acting by cleavage of specific HCV-RNA sequences. Heptazyme is a ribozyme against the HCV IRES, which had progressed to early phase clinical studies in patients with CHC. It showed moderate anti-viral efficacy, but further development was stopped because of toxicity in animal models.

V-Inhibitors of viral assembly and release: [104]

Inhibitors of cellular glycosidases are potential candidates of anti-viral therapy, inhibiting viral assembly and release. Celgosivir or MX-3253 is an inhibitor of the host enzyme a-glucosidase I, which is involved in HCV assembly and release.

VI- Prevention of binding: [104]

The E1 and E2 are type I transmembrane highly glycosylated proteins of the HCV envelope, and E2 is considered to play an important role in viral attachment interacting with one or more components of the cell membrane, such as low-density lipoprotein receptor, glycosaminoglycans and CD81. The anti-viral activity of specific E2-derived peptides, such as GNS-037, have been evaluated in vitro with encouraging results.

Improvements in Current Therapies: [104]

*1)**Consensus interferon*** *(*recombinant, non-naturally, 166-amino acid IFN, containing the most frequently observed amino acids from various natural ones*)*, ***Albuferon*** *(*IFNa genetically fused to human albumin extending its serum half life, supports dosing at 2- to 4-week intervals*)*, ***Omega interferon*** *(*new type-1 IFN, homologous to IFNa in 60% and homologous to IFNb in 30%, that has been designed for continuous delivery by an implantable device), ***Interferon lambda*** (Uses a different receptor that is mainly present in the liver, with limited distribution elsewhere in the body improving tolerability compared with interferon alfa: less hematologic toxicity , fewer musculoskeletal and flu-like symptoms).

2) *Ribavirin analogues:* tolerability is freqently limited because of RBV induced haemolytic anaemia. ***Viramidine or taribavirin*** *(*prodrug that is metabolized preferentially in the liver by adenosine deaminase and thus it does not accumulate in erythrocytes), ***VX-497 or Merimepodip***(potent, specific and orally taken inhibitor of IMPDH).

Strategies for Prevention

HCV-prevention programs are needed at the local, national, regional and global levels if the spread of HCV and the burden of hepatitis C are to be reduced. To achieve these objectives, the implementation of measures that reduce the risk of contracting HCV infection is required, a task that was unfulfilled in many areas in 2010. Such programs need to ensure that blood supplies and related products are free of infection, and that safe injection methods are practiced within and outside medical settings. The use of disposable syringes for immunization and injections is particularly crucial in developing countries. Risk-education counseling for professionals and the public is of paramount importance. Where this is affordable, persons with chronic hepatitis C should be identified and targeted for special counseling and medical management, in order to reduce the risk of them developing HCV-related disease complications. (1, 58, 92)

Healthcare professionals and the public, who are crucial for the effective prevention of HCV transmission, should be educated about the risk of transmission of blood-borne pathogens (HCV, hepatitis B virus and HIV) by contaminated injection and other medical equipment, as well as by traditional and folk medical procedures or practices. They should receive appropriate education and training concerning the importance of controlling such infections in all medical, surgical and dental facilities, including the use of standard precautions, safe injection practices, proper sterilization techniques, and high-level disinfection where appropriate, avoiding the re-use and sharing of contaminated equipment and supplies, and avoiding contamination of multi-use supplies, such as medication vials. The use of devices or products that prevent re-use or contamination of medical and dental equipment should be encouraged (e.g. autodestruct syringes), noting that cost-effective devices are available.(14, 79)

Vaccine Development

As HCV infection has major public health implications, the development of an effective vaccine is of paramount importance. However, such an effort is not without daunting challenges. First, the virus exists as a quasi-species because of a high rate of mutation in the hypervariable region of the envelope proteins. Second, the hypervariable region is a major site of antienvelope antibody response and contains a principal neutralization epitope. Third, antibody responses to the envelope proteins develop slowly and achieve only modest titers during primary infection. Consequently, neutralizing antibodies may emerge too late to prevent chronic infection. In addition, antienvelope antibodies tend to be short-lived, disappearing gradually after viral clearance. Fourth, immunologic correlates of protection and disease progression have not been clearly defined.(105, 106)

These problems are further complicated by a lack of a convenient infectious tissue culture system for testing neutralizing antibodies or passage of attenuated viral strains. In addition, the only infectious animal model is the chimpanzee, an endangered species that is difficult to study; in addition, the course of HCV infection in the chimpanzee is not necessarily representative of that in humans.(105, 106)

The ideal HCV vaccine should elicit high-titer, long-lasting, and broadly directed antienvelope antibodies that recognize conserved epitopes and neutralize against all HCV isolates. The vaccine should also be capable of inducing a vigorous, multispecific cellular immune response that includes both helper and cytotoxic T lymphocytes. In particular, conserved T-cell epitopes in the core, NS3, and NS4 regions should be targeted. Finally, because the Th1 response is important in viral clearance, a vaccine candidate should direct a predominantly Th1 response.[22, 107-109]

Several approaches have been used to develop an HCV vaccine. The classic approach of developing live attenuated viral strain is hindered by the lack of convenient experimental systems. The initial effort was directed toward generating recombinant HCV envelope proteins as a subunit-based vaccine, but success was limited. Immunization of chimpanzees with the subunit vaccine resulted in partial and transient protection against low-dose challenge of a homologous, but not heterologous, strain. Genetic vaccination holds great promise for induction of broadly directed humoral and cellular immune response; however, preliminary experiments in chimpanzees demonstrated that DNA immunization with HCV gene constructs may not be particularly immunogenic. Furthermore, the development of chimeric viruses expressing HCV gene products is attractive, but safety and regulatory issues may surface with implementation. An alternative approach relies on the synthesis and production of virus like particles. In contrast to the recombinant subunit-based vaccine, the structural proteins of HCV-like particles are presented in a native, virion-like conformation and may therefore be superior in eliciting a protective immune response. In addition, HCV-like particles, as a particular antigen, may elicit a cytotoxic T-cell response, which plays a critical role in viral clearance.[110-112]

Approaches of vaccination:

1. *Prophylactic vaccination:*

Early attempts focused on inducing the production of neutralizing antibodies against envelope proteins of HCV, E1 and E2. This was inspired by the success of HBV vaccines, which induce antibodies against HBV surface antigens, thereby preventing viral entry and infection. Induction of HCV envelope-specific antibodies in naive chimpanzees by vaccination with recombinant E1 and E2 or DNA, yielded protection from virus challenge. Similarly, immunization of healthy human volunteers with HCV envelope glycoproteins elicits antibodies that crossneutralize heterologous virus strains *in vitro*. A major challenge remains in the identification of suitable immunogens that elicit broadly neutralizing antibody responses. The major antigen determinants within the viral envelope are in the hypervariable-region 1 of the E2 glycoprotein, which, as the name implies, is not necessarily suitable to confer broad protection against antigenically diverse viruses. It has been speculated that more broadly shared epitopes will become accessible when the HVR1 region is deleted from the viral envelope. However, the idea of engineering the immunogenicity of HCV by exposing better-conserved epitopes remains to be tested. Furthermore, analysis of the structural details of (conformational) epitopes recognized by antibodies with broad neutralizing activity may provide a starting point for the design of immunogens capable of eliciting antibodies with similar activity.

Prophylactic vaccination approaches are not limited to those geared towards inducing neutralizing antibodies. Clinical trials are ongoing to assess the efficacy, safety and immunogenicity based on the sequential use of adeno- and/or modified vaccinia Ankara (MVA) vectors expressing HCV nonstructural proteins NS3-NS5B. Conceivably, combining the approaches that prime both humoral and cellular immunity would protect more efficiently against HCV challenge, although the concept remains to be tested in suitable animal models and/or clinical trials.[111, 113, 114]

2. Therapeutic vaccination:

The main rationale of therapeutic vaccination is to booster new and restore ineffective previously primed antiviral adaptive immune responses to neutralize circulating virus and eliminate infected cells. Optimally, therapeutic vaccination, conceivably in combination with standard-of-care treatment, would eventually result in complete control of the previously established viral infection or at least significantly halts liver disease progression. Treatment of chronic HCV infection has considerably improved in recent years and numerous directly acting antiviral and host-targeting antiviral drug candidates have shown remarkable efficacy in clinical trials. These advances may ultimately reduce the need for therapeutic vaccines.[115]

Therapeutic vaccine trials have demonstrated that HCV-specific immune responses can be primed in chronically infected individuals, resulting in transient reductions in HCV RNA titers in subsets of patients. However, to date, no therapeutic vaccine candidates have achieved sustained SVRs. Considering that immune exhaustion is frequently associated with chronic HCV infection, the fact that partially functional T-cell responses can be primed is still remarkable. These observations also argue that a better understanding of mechanisms of immune exhaustion is needed to pair therapeutic vaccinations with specific immunomodulatory regimens to booster antiviral immunity.

Multiple approaches has been undertaken towards developing a therapeutic vaccine against HCV infection. These can be broadly divided into peptide- or protein-based vaccines, DNA vaccines, viral vector vaccines – including recombinant adenovirus, MVA, alphavirus or paramyxovirus vectors – recombinant yeast-based vaccines and vaccination approaches based on dendritic cells (DCs). Of those, some have advanced into early clinical development assessing their safety and immunogenicity, but only few are currently being actively pursued. For example, it was previously demonstrated that HCV antigen expression from DNA can result in robust induction of HCV-specific humoral and T-cell immunity, depending on the antigen combination. Currently, administration with a plasmid expressing NS3/4a of HCV genotype 1 and subsequent *in vivo* electroporation is being tested in combination with peg-IFN and RBV in chronically infected HCV patients. In contrast to plasmid DNA vaccines, viral vectors are highly immunogenic and also allow for the expression of an antigen combination of choice. From a regulatory perspective of safety, insufficient or incomplete attenuation of replication of viral vector is a major concern. Replication incompetent adenoviral and MVA vectors have been extensively tested in this respect. To induce anti-HCV immunity, adenoviruses alone and/or with MVA expressing HCV nonstructural proteins in combination with standard-of-care therapy are currently being evaluated for their potential to restore dysfunctional T-cell response and to broaden HCV-specific T-cells' immunity.[10, 110, 111]

Glucose abnormalities in hepatitis C virus infection:

HCV is both hepatotropic and lymphotropic. Replication of HCV in diseased extrahepatic organs and tissues may either trigger latent autoimmunity or induce autoimmune disorders. In addition to established liver injury, type 2 diabetes mellitus (T2DM) is an important feature of extrahepatic metabolic disorders which is attributed to HCV infection. It also has some impact on the disease activity, disease course, clinical outcomes, and treatment efficacy of antiviral therapy. Previous experimental and clinical findings have highly suggested that HCV *per se* is diabetogenic. The cause–effect interaction between a common endocrine disorder and an infectious disease is an important issue to elucidate. Although the precise mechanisms whereby HCV infection leads to insulin resistance (IR) and glucose abnormalities are not entirely clear, it differs from the usual pathogenesis of T2DM in those with non-HCV liver diseases.[116-119]

The liver has long been regarded as the key player manipulating the homeostasis of glucose metabolism. As the largest reservoir of glucose, the liver's role in glucose metabolism draws much attention in patients with advanced liver disease. Insulin resistance is therefore a common feature of some liver diseases, especially at advanced stages. Hepatic diabetes was recognized when diabetes developed in patients who had advanced liver cirrhosis or severe liver injury, in which overt fasting hypoglycemia and/or postprandial hyperglycemia emerge as a common phenomenon. However, the association between T2DM and CHC is beyond the concept of hepatic diabetes. Although type 1 DM has been observed in patients who were treated with interferon (IFN), the majority of HCV-related diabetes is T2DM.[120-123]

Epidemiological view

The association between T2DM and CHC was first reported in 1994 by Allison *et al*, who observed that the prevalence of T2DM was significantly higher in those with HCV-related cirrhosis than those with cirrhosis resulting from other liver diseases. The diagnosis of HCV infection and the identification of risk factors for HCV infection preceded the diagnosis and/or onset of T2DM in anti-HCV positive diabetic patients. Generally, the prevalence of anti-HCV seropositivity in the T2DM population ranged from 1.8% to 12.1%, whereas T2DM developed in 14.5–33.0% of CHC patients. Different background in terms of ethnicity, age, prevalence of T2DM, body mass index (BMI), viral load and genotype may contribute to the divergent results of the epidemiological observations.[117, 118]

Studies extended the observation that HCV viremia, but not anti-HCV seropositivity alone, increased the association with T2DM. HCV viremia was the most significant independent factor to be associated with T2DM, followed by well-established factors such as male gender, hypertension, BMI and age. It may imply that a persistent and/or active phase of HCV infection is associated with T2DM. Taken together, a link between CHC and T2DM has been supported by few epidemiological evidence. T2DM represented one more disease to be included in the list of established extrahepatic manifestations of HCV infection. The relationship between T2DM and HCV genotypes remains controversial. It was demonstrated that HCV genotype-2a (G-2a) was specifically linked with extrahepatic manifestations such as cryoglobulinemia. An association between G-2a infection and T2DM was also shown.[117, 118]

Pathophysiological view: (Figure 10)

T2DM is a common endocrine disorder encompassing multifactorial pathogenic mechanisms. These mechanisms include resistance to the action of insulin, increased hepatic glucose production, and a defect in insulin secretion, all of which contribute to the development of overt hyperglycemia. As well as skeletal muscle and adipose tissue, liver is the major target for the metabolic actions of insulin. Insulin regulates glucose homeostasis by reducing hepatic glucose output and by increasing the rate of glucose uptake by skeletal muscle and adipose tissue. Therefore IR is a common feature of advanced liver diseases from various insults.[124-126]

HCV has been shown to be a lymphotropic as well as a hepatotropic virus. Replication of HCV in diseased extrahepatic organs and tissues may have cytopathic effects. The precise biological mechanisms whereby HCV infection leads to IR are not entirely clear. HCV may induce a Th1 lymphocyte immune-mediated response which leads to activation of the tumor necrosis factor (TNF)-α system and elevation of interleukin-6 levels. A high TNF-α level was considered to be one of the bases of IR, which act by disturbing tyrosine phosphorylation of insulin receptor substrate (IRS)-1, a central molecule of the insulin-signaling cascade. Meanwhile, HCV directly causes liver steatosis. All the above events may precipitate the development of liver fibrosis. [118, 127, 128]

TNF-α system activation, liver steatosis and fibrosis contribute to the development of IR, which plays a pivotal role in the development of subsequent metabolic events. Meanwhile, HCV-induced inflammatory changes may subsequently lead to increased oxidative stress and increased peroxidation, which evoke systemic inflammatory responses (SIR) more often than other liver diseases. SIR triggered by HCV and/or its subsequent immune cascades and cytokine storms may play a major role in the related pathogenic mechanisms in terms of liver injury and the unique extrahepatic manifestations. SIR may also contribute either directly or indirectly to the disease course, viral response, disease severity, and response to antiviral treatment. Cytokine triggering, which interacts with innate and/or adaptive immune responses, is one of the major concealed players of the scenario.[129-131]

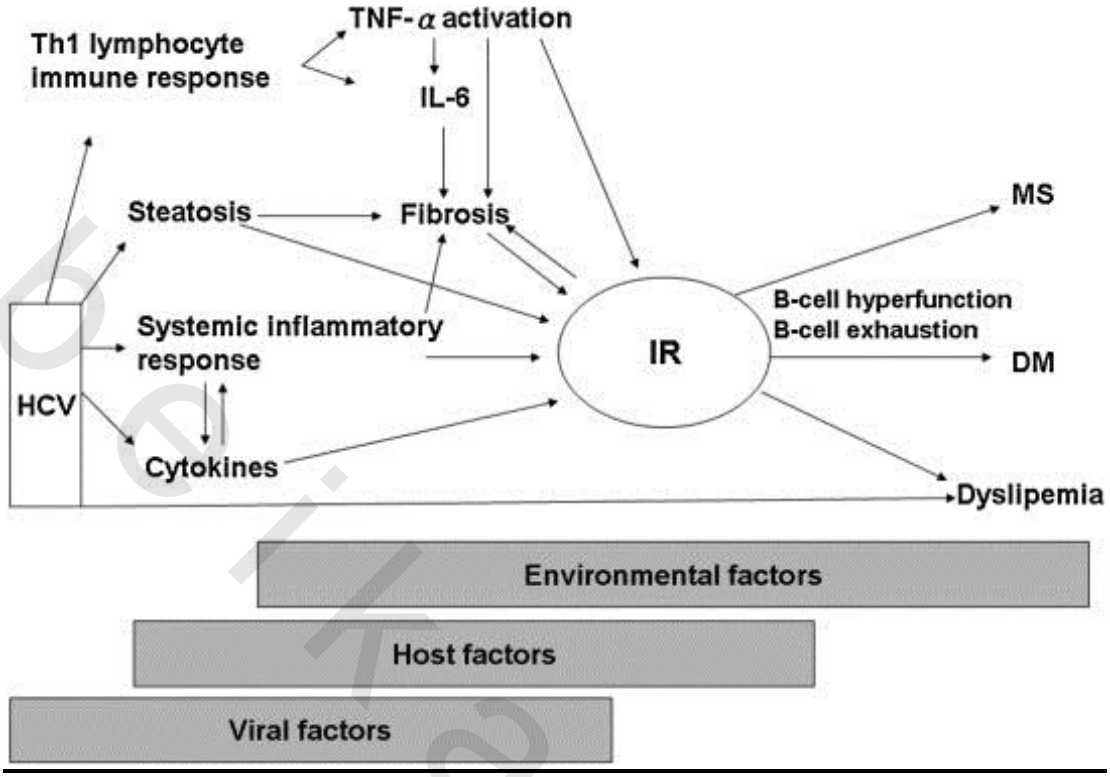

Figure 10: Pathophysiology of development of insulin resistance in HCV patients.

Evidence showing a direct diabetogenic effect of HCV *per se* came from previous experimental study showing that the ability of insulin to lower the plasma glucose level was impaired without gain in body weight at young age in HCV core gene transgenic mice. HCV core-induced suppressor of cytokine signalling 3 (SOCS-3) may promote proteosomal degradation of IRS1 and IRS2 through ubiquitination, which may be a unique mechanism of HCV-associated IR. In a clinical observation, an increase in fasting insulin level was associated with the presence of serum HCV core protein, the severity of hepatic fibrosis, and a decrease in expression of IRS-1 and IRS-2 in patients with HCV infection. More severe IR was present in noncirrhotic patients with HCV infection than in patients with other liver diseases. Taken together, HCV core protein seems to play a pivotal role in HCV-associated IR. The precise mechanisms whereby HCV infection leads to IR and glucose abnormalities may differ from the usual pathogenesis of T2DM in those with non-HCV liver diseases. HCV may induce IR regardless of the severity of liver disease and IR may be associated with severe hepatic fibrosis and contribute to fibrotic progression. There was also a dose–response relationship between HCV RNA level and the presence of IR, whilst IR was positively associated with the severity of hepatic steatosis.[132-134]

Clinical view:

The "gold standard" for IR assessment is the hyperinsulinemic–euglycemic clamp test. However, the difficulty of the technique and its laborious nature much discourage the wider use of the test. The Homeostatic Model Assessment (HOMA), which has been used in large epidemiological studies, offers an estimate of IR. HOMA has the advantage of requiring only a single fasting plasma sample measured for glucose and insulin. It therefore provides a wide scope of view addressing the correlation between HCV infection and IR. Several clinical predictors of the sustained virologic response (SVR) to combination therapy have been elucidated. The viral factors include genotypes, pretreatment viral load and on-treatment viral kinetics, whereas the host factors constitute age, BMI, races and interleukin-28B (IL-28B) polymorphisms. [118, 124, 135]

Glucose abnormalities have also been suggested recently to be a risk factor for nonresponse. Patients with high HOMA-IR achieved a significantly lower rate of SVR than those who with low IR. The significantly lower SVR rate in high HOMA-IR patients compared to low HOMA-IR patients was observed in G-1 patients but not in non-G-1 patients. Of note was that IR was associated with SVR, especially among "difficult-to-treat" patients, i.e., the patients with G-1 infection and high pretreatment viral loads (>400,000 IU/mL). Since IR is considered as a factor which can be modified and improved by various interventions, further prospective studies will be valuable to evaluate whether the effective approaches to improve IR before initiation of the combination therapy for CHC can significantly increase the SVR rate.[118, 130]

Whether or not reducing IR using insulin-sensitizing agents improves treatment outcomes is speculative. Current data from clinical trials by adding pioglitazone or metformin have proved to be disappointing. This may suggest that the emergence of IR in CHC evolved from the multifactorial, complex context encompassing viral, host and environmental factors.[129, 136]

Treatment outcome affecting glucose abnormalities

IFN has been used widely for CHC treatment for two decades. However, IFN is an integral player in immunity and may exacerbate an existing autoimmune tendency, which may subsequently precipitate immune-mediated abnormalities. Emergences of IR and subsequent DM have been demonstrated with IFN-based therapy, although the mechanism remains to be clarified. Therefore, the interplay between IFN-based antiviral therapy and alteration of insulin sensitivity deserved to be elucidated. Reduced IR and subsequent improved glucose control after conventional IFN therapy was observed among chronic hepatitis B or CHC patients. It was further demonstrated that clearance of HCV improves IR, beta-cell function, and hepatic IRS1/2 expression by immunostaining, whilst there were no significant changes in IR and beta-cell function after antiviral therapy in non responders and relapsers. [137, 138]

The significant decline of HOMA-IR after treatment was observed only in patients with high pretreatment HOMA-IR, regardless of SVR achievement. Studies of CHC patients showed that SVR was independently associated with a reduction in IR in G-1 but not G-2/3 patients. The results suggest a causal relationship between specific genotype and IR. The somewhat discordant results may imply that the HOMA-IR with respect to SVR may have been influenced by variables such as race, age, genotypes, validation methods for diabetes, cut-off value of IR, treatment adherence, and/or the presence of liver steatosis. Since the mechanisms that are involved in the emergence of IR are multifactorious, further long-term follow-up study is needed to elucidate them in this context.[139-141]

In addition to hyperinsulinemia, pancreatic beta-cell hyperfunction aiming to maintain glucose homeostasis and elevated serum insulin level is the main feature of glucose abnormalities. The scene is also common in HCV infection, and insulin secretion is increased in the initial stages of HCV infection to compensate for IR development in both experimental and human studies. The sequential change of beta-cell function might suggest that beta-cell function recovered earlier than that of IR in CHC patients receiving PegIFN/RBV combination therapy.[136, 142, 143]

However, management of IR mainly depends on both pharmaceutical intervention and lifestyle modifications, such as exercise, diet control and weight reduction. Whether these interventions play a role in the disease course and prognosis of CHC patients deserves to be elucidated.[131, 144, 145]

Interaction with IL-28B genetic polymorphism:

Recently, studies based on genome-wide associated studies (GWAS) have shown that single nucleotide polymorphisms (SNPs) at and/or near the *IL28B* gene, which encodes interferon-λ, play a critical role in the treatment efficacy of HCV infection. It has been considered to be the most important host factor contributing to SVR. Therefore, the interplay between *IL28B* polymorphism and IR and its related outcomes after antiviral therapy in CHC patients deserves to be elucidated.[85, 146]

Recent studies demonstrated that there was no significant difference in *IL28B* genotype distribution according to pretreatment IR. IR may undermine the advantages of a favorable *IL28B* polymorphism to achieve SVR in G-1 patients. There was no significant association between *IL28B* gene polymorphism and baseline IR across G-1 and -2 patients. However, discordant results were recently reported from another European study showing that IR is more common in carriers of the unfavorable allele of *IL28B* in G-1 and -4 nondiabetic patients.[85, 146]

However, no significant association was observed between *IL28B* gene polymorphism and outcome of glucose abnormalities after completion of treatment. Despite no statistical significance being reached, the prevalence of a favorable gene polymorphism in patients who developed DM after therapy tended to be lower than patients who remained as prediabetic and those who developed normoglycemia after therapy. The real scenario between gene polymorphism and IR in different phases of glucose abnormalities deserves to be further evaluated in a long-term fashion.[85, 146]