Hepatitis C virus

I.A brief history of hepatitis C virus:

The hepatitis C virus (HCV) was first identified in 1989 by immuno-screening an expression library with sera from patients with post-transfusion non-A, non-B hepatitis (NANBH) [(1)]. The complete HCV nucleotide sequence was quickly established and allowed the classification of this new agent into the Flaviviridae family [(2)]. Flaviviridae are enveloped Ribonucleic acid (RNA) viruses and include yellow fever virus, dengue virus and tick borne encephalitis virus, all of which cause disease in humans [(3)]. HCV is the unique member of the Hepacivirus genus within the Flaviviridae family [(4)]. Amongst Flaviviridae, the GB viruses (hepatitis G virus (HGV)) are the closest HCV relatives with Hepatitis GB virus B (GBV-B) sharing some characteristics with HCV in its infection profile [(5)].

HCV is an enveloped flavivirus with a 9.6 kilo base (kb) single-stranded RNA genome [(6)]. Based on the characteristics of the nucleotide sequence the NANBH virus contains a 10,000 nucleotide positive-strand RNA genome consistent with members of the Togaviridae and Flaviviridae families - the new virus was classified within a separate genus (Hepacivirus) of the Flaviviridae family and designated Hepatitis C Virus (HCV) [(7)].

II. Epidemiology:

Chronic hepatitis C virus (HCV) infection affects more than 200 million persons worldwide and responsible for the development of liver diseases in many cases [(8)].

Worldwide, it is estimated that 170-200 million people are living with chronic hepatitis C infection (~3% of the world's population), that it infects 3-4 million people per year, >10% of these people will develop liver cirrhosis or cancer over time and that more than 350,000 people die from hepatitis C related diseases each year [(9)].

Egypt has the highest hepatitis C virus (HCV) prevalence worldwide; with an estimated overall prevalence of 21.9% among adults in 1995-1996 [(10)].This is markedly higher than in industrialized countries where the prevalence ranges from 0.5% to 2.3% [(11)]. It is also higher than in limited resource countries, even those which reported high prevalence rates such as Pakistan (6.5%) and Mongolia (15.6%) [(12, 13)].

While, there are numerous studies documenting the emergence of HCV as an important public health problem in developing countries, there are few studies characterizing the epidemiology of HCV transmission in those countries. While transmission associated with injection drug use is also a well-recognized risk factor, many studies suggest that most HCV infections in the developing world are attributable to health care-related exposures [(14)].

Primarily HCV is spread by direct contact with human blood. Risk factors most frequently associated with HCV transmission are transfusion of unscreened blood, injection drug use, unsafe injections, and other iatrogenic health care procedures. The development of diagnostic assays to screen blood and blood products has resulted in a remarkable reduction in transfusion-associated HCV infection in many developed

countries [15]. Similarly, the implementation of standard precautions to prevent occupational exposure to blood has resulted in a decrease in blood-borne pathogen transmission in the health care setting [16]. Since the implementation of these measures, epidemiologic studies indicate that injection drug use is the predominant mode of transmission in developed countries [17].

HCV transmission in Egypt has been the subject of intense study in the two decades since HCV diagnostic assays became available. Several studies have documented a prevalence of HCV antibodies ranging from 6 to 20% in the general population [18]. It is generally accepted that the high prevalence of HCV infection is in part attributed to parenteral anti-schistosomal treatment campaigns that were conducted in the 1970s. However, epidemiologic studies suggest that HCV continues to be transmitted at relatively high rates [19]. Monitoring the incidence of HCV infections is difficult because most infections are asymptomatic and available assays do not distinguish acute from chronic or resolved infections [20].

Prospective cohort studies monitoring HCV sero conversion among susceptible persons have identified incidence rates ranging from 3.1 to 5.2 per 100,000 [21]. Based on these studies, it is projected that 248,000 to 416,000 infections may occur each year in Egypt [22].

Numerous epidemiologic studies have reported diverse exposures associated with HCV infection in Egypt, including unsafe injections, health care procedures, and various community exposures. However, there are limitations to the interpretation of these data since many are based on HCV-infected persons identified in cross sectional sero-prevalence surveys and not among patients with newly acquired infections. Without well-designed studies of patients with acute disease, it is challenging to develop prevention strategies that are focused on the most important sources of ongoing transmission. That study is designed to identify risk factors among patients with newly acquired HCV infection, focusing on various healthcare and community-level practices. It is anticipated the results will inform health officials on key areas to formulate strategies for the prevention of HCV transmission in Egypt [23].

It has been reported the prevalence of chronic HCV infection in Egypt is higher among men than women (12% and 8%, respectively), increases with age (reaching >25% among persons aged >50 years), and higher among persons residing in rural versus urban areas (12% versus 7%) [24].

Further, with such a high background prevalence rate, transmission of hepatitis C through other nonmedical routes has become more significant in Egypt e.g., Tattooing, circumcision and other medical procedures performed by non-medical persons. In addition, household transmission, vertical transmission and sexual transmission are routes that are also under investigation [25].

III. Molecular aspects of Hepatitis C Virus infection

III.A. Model structure of HCV:

III.A.1. The genome:

HCV virions exhibit a wide range of densities, although the most infectious fraction has a density of 1.15-1.17 gram per millimeter (g/ml) [26]. Present inside the outer envelope, there is a 30-35 nanometer (nm) inner core which encapsulates the single-strand viral RNA (positive-sense), which is approximately 9.6 kilo base (kb) Figure(1) [27].

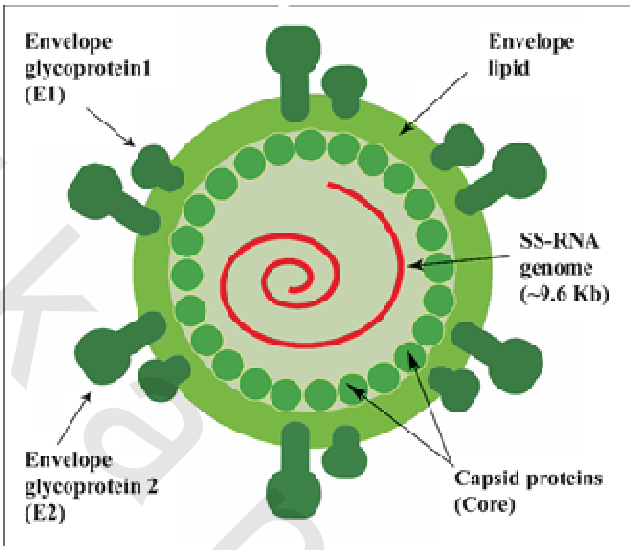

Figure (1). Heptiatis C virus particle structure: The HCV core protein interacts with viral genomic RNA to form the nucleocapsid [28]. Two membrane-associated envelope glycoproteins, E1 and E2 are embedded in a lipid envelope which is derived from the host [29].

The HCV genome does not enter the cell nucleus, thus, HCV- RNA replication occurs in the cytoplasm of hepatocytes. The viral-RNA genome harbors a single ORF (open reading frame) which is flanked by 5' and 3' non translated RNA segments (NTRs). The cis-acting replication elements or CREs are located in both the 5' and 3' NTRs and in the nonstructural proteins (NS5B) coding sequence[30].

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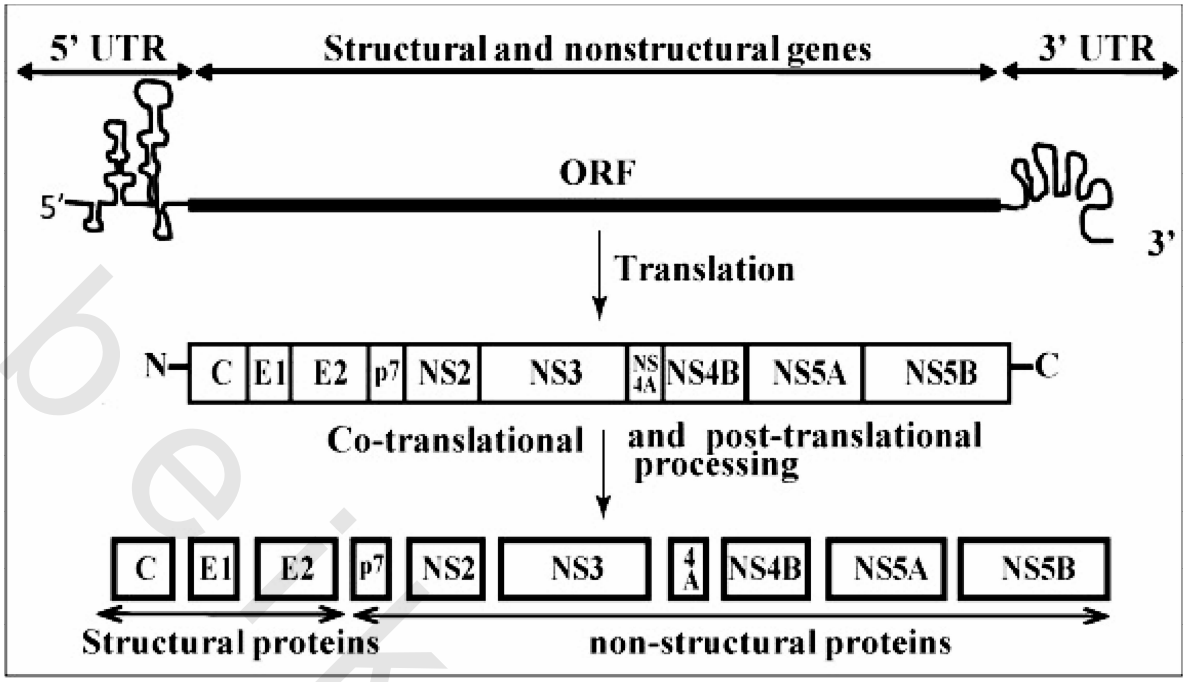

Figure (2). A schematic representation of HCV genome, structural and nonstructural proteins: The viral RNA consists of a 5' untranslated region (UTR) containing the internal ribosome entry site (IRES), followed by the genomic region for structural and nonstructural genes and the 3' U TR. HCV is translated as a polyprotein that is proteolytically processed by host and viral proteases[31]. The structural proteins (C, core; E1, envelope protein 1; E2, envelope protein 2) are located at the amino-terminal end while the nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are located at the carboxyl-terminal end70. The p7 protein (ion channel or viroporin) is located at the junction of structural and nonstructural proteins[31].

The 5'- and the 3'-NTRs of the genome are highly conserved and contain control elements for translation of the viral polyprotein and replication. Untranslated region (UTR) the 5′ UTR (+) is ~341 nucleotides in length and contains an internal ribosomal entry site (IRES). The HCV IRES is folded into four stem-loop motifs which are called as I, II, III and IV. The IRES is required for cap-independent translation of viral RNA, which is carried out by host cell ribosome. The domain III d of the IRES constitutes the key anchoring site for the 40S subunit [32]. The IRES domains III-IV have also been shown to be an activator of protein kinase R (PKR) [33]. However, this activation does not interfere with cap-independent translation of HCV viral proteins. HCV core protein was reported to interact with the 5'-NTR of plus-strand RNA [34].

The 3'-UTR (+) is around ~200 nucleotides (nt) and is involved in RNA replication. Three different domains can be recognized in this UTR : (i) a poly Uracil/Uracil C (U/UC) tract with an average length of 80 nucleotides (nt), (*ii*) a variable region, and (*iii*) a virtually invariant 98-nt X-tail region made up of 3 stem-loops (3'SLI, 3'SLII and 3'SLIII). The 3'-UTR can robustly stimulate IRES dependent translation in human hepatoma cell lines [35]. Recent studies have recognized that various stem-loop structures

exist in the negative strand 3'-NTR. This region is recognized by the viral polymerase as the initiation site for plus-strand synthesis of the HCV genome [36].

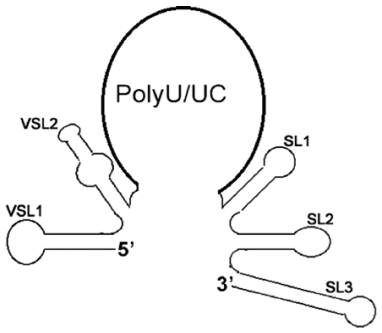

Figure (3). The predicted secondary structure of HCV 3′-NTR. [37] (i) An upstream variable region folded into two stem-loop structures designated as VSL1 and VSL2. (ii) A large poly (U)/UC tract. (iii) A highly conserved 98-nucleotide 3`-terminal segment that forms three stem-loop structures designated as SL1, SL2, and SL3.

Non coding RNA molecules or microRNAs (miR) are important in the control of gene expression and regulation. MicroRNA, miR-122 is specifically expressed and is found to be abundant in the human liver [38]. A recent discovery showed binding of a miRNA (miR-122) to the 5'-UTR of HCV. Sequestration of miR-122 in liver cell lines strongly reduced HCV translation, whereas its addition stimulated translation via direct interaction of miR-122 with two sites in the 5'-UTR [39].

III.A.2. HCV Structural Proteins:

HCV encodes a single polyprotein (NH2-C-E1- E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH) which is approximately 3010 amino acids (Fig. 2). The structural proteins (core, E1 and E2) and the p7 protein are released from the polyprotein after cleavage by host endoplasmic reticulum (ER) signal peptidase(s)[27].

III.A.2.i. Core:

HCV core is a multifunctional protein which is highly basic in nature [40]. It forms the structural component of the virus particle. Core has been implicated in the development of hepatocellular steatosis and oncogenesis. The core protein is generated from a polyprotein encoded by the viral genome and is processed by cellular proteases in the endoplasmic reticulum (ER) [41]. It is essential for association, not only with the ER but also with the outer mitochondrial membrane[42]. The HCV core protein is known to pass from the ER into mitochondria and is involved with ionized Calcium (Ca^{2+}) regulation and apoptotic signals [43].

Core has many intriguing regulatory functions with one of the most important being recruitment of non-structural proteins to the lipid droplet-associated membranes [44]. Lipid droplets (LDs) are intracellular organelles involved in lipid storage and also take part in intracellular vesicular trafficking [45]. HCV makes use of lipid droplets for replication. In infected Huh-7 cells, the core protein is associated with the surface of lipid droplets [46].

Besides its structural and regulatory function, core plays an imperative role in the pathogeneses of liver steatosis. Up regulation of *de novo* fatty acid biosynthesis by HCV core protein in Huh7 cells has been reported. Core protein also interacts with Apo lipoprotein AII, a component of lipid droplets. HCV core protein is targeted to lipid droplets by its domain 2 (D2) and this association with lipid droplets is required for virus production. Disrupting the association of core protein with lipid droplets is deleterious for HCVcc production and this interaction is thought to contribute to steatosis, via deposition of triglycerides in the liver [(45)].

In the cytoplasm the core protein is mostly localized to the endoplasmic reticulum (ER). HCV core protein, circulating 'free' in non-enveloped state has also been detected in HCV infected patients [(46)].

III.A.2.ii. Envelope glycoproteins:

The HCV envelope glycoproteins, E1 and E2 are structural components of the virion. They constitute the outer coat of fine spike like projections of the HCV particle (Fig. 1) [(47)]. They undergo post-translational modifications (N-linked addition of carbohydrate chains) while being translated in the endoplasmic reticulum (ER). Both, E1 and E2 envelope glycoproteins are required for host-cell entry via receptor binding. Insight into the mechanisms by which HCV gains entry into host cells is vital to understand primary HCV infection and re-infection post-transplantation [(27)].

The E2 protein has the binding sites for human Cluster of Differentiation 81 (CD81), a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes [(48)]. The binding of E2 was further mapped to the major extracellular loop of CD81. CD81 along with human scavenger receptor SR-BI, and tight junction molecules claudin-1 (CLDN) and occludin (OCLN) are the most important receptors that mediate HCV entry[(49)].

HCV pp system (also called as HCV pseudo particle) generates virus particles, which display E1-E2 glycoproteins of HCV on their surface. Cell entry of HCVpp is HCV glycoprotein mediated. It is interesting to note that HCV can associate with Low density lipoprotein (LDL) and Very Low density lipoprotein (VLDL) from infected patient serum. Amazingly, HDLs play a very active role in HCV entry. A complex interplay between high density lipoprotein (HDL), Sterol regulatory element-binding protein-1(SRBI) and HCV envelope glycoproteins leads to enhanced HDL-mediated HCVpp entry in cells [(50)]. In addition, HDL can inhibit HCV-neutralizing antibodies in serum of acute and chronic HCV-infected patients[(51)].

III.A.2.iii. The p7 ion channels:

The HCV p7 is a small 63 amino acid protein, positioned at the junction of the structural and non-structural proteins. The p7 protein belongs to a family of viral proteins called as viroporins that form ion channels. It can oligomerize following its inclusion into a lipid membrane creating ion channels. The cleavages mediated by signal peptidases between p7 and NS2 occur slowly and are partial at the E2/p7 and p7/NS2 sites [(52)]. This results in the formation of an E2p7NS2 precursor [(53)].

It is thought that this precursor may have a role in the regulation of HCV lifecycle. The p7 protein is highly hydrophobic in nature. It is localized in the ER-membranes when encoded by a replication-competent genome [54]. It has two amphipathic, Trans membrane regions, TM1 and TM2 (spanning amino acids 19-32 and 36-58) which are embedded in the ER-membrane. The N- and C-termini are exposed to the extracellular environment [55].

The p7 ion channel protein serves an essential role in the production of infectious virus particles during HCV lifecycle [56]. It appears to be dispensable for viral RNA replication, as replicons lacking the p7 gene replicate or make viral RNA efficiently [57].

III.A.3.HCV Non Structural Proteins:

The non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are cleaved by viral proteases NS2-3 and NS3-4A. This proteolytic processing of the polyprotein during and after translation by host and viral proteases yield at least 10 mature viral proteins. The non-structural proteins are thought to be to be required for replication of the viral genome. The non-structural protein, p7, can form ion channels, required for the production of infectious virus particles. It is now recognized that the cross-talk between structural and the non-structural proteins of HCV is required for efficient virus particle production [58].

III.A.3.i. HCV NS2:

The nonstructural protein 2 (NS2) is a 23-kDa (kilo Dalton) Trans membrane hydrophobic protein. The membrane association of NS2 is p7-independent and occurs co-translation ally [59]. NS2 is a membrane associated cysteine protease [60], required for HCV in cell culture (HCVcc) infectivity [61].The cleavage between NS2 and NS3 is absolutely required for persistent viral infection in a chimpanzee [62]. NS2 followed by only 2 amino acids of NS3 produces a basal proteolytic activity *in vitro*. However, the N-terminal 180 aa (amino acid) of NS3 are required besides NS2 for a robust protease activity. Interestingly, all active site residues (H952, E972 and C993), needed for the catalytic activity of the NS2/3 cysteine protease, are located entirely in NS2 [63].

This requirement of NS3 remained intriguing until the recent discovery showing that the zinc binding domain of NS3 could in fact stimulate the protease activity of NS2. The functional sub-domains in NS3 essentially function as its regulatory cofactor [63] again highlighting tight regulation of the proteolytic processing. This process is undoubtedly vital for virus multiplication. NS2 interacts with itself forming homo-dimers. Moreover NS2 has also been shown to interact with all the other HCV nonstructural proteins [64].

Until recently the only known function for NS2 was its auto cleaving activity at the NS2/3 junction. Expression of NS2 in Huh7 cells resulted in up regulation of transcriptional factor sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase. These studies implicate a role of NS2 in promoting steatosis. NS2 protein can be phosphorylated by casein Kinase II on Ser residue at position 168. This phosphorylation event appears to regulate the stability of NS2 protein (at least from genotype 1a). NS2 also appears to be involved in a particle assembly step which happen post core, NS5A, and NS3 assembly [65].

III.A.3.ii. HCV NS3:

The non-structural protein 3 (NS3) is a member of the superfamily 2 DExH/D-box helicases and its crystal structure has been determined. It is a 67 kDa tri-functional protein with a serine protease, an RNA helicase and Nucleoside triphosphatases (NTPase) activities. The NS3 enzyme has a chymotrypsin-like serine protease activity [66]. Along with its cofactor NS4A is responsible for cleavage at the NS3/4A, NS4A/4B, NS4B/5A and NS5A/5B junctions. NS3/4A is localized in ER cisternae surrounding mitochondria[67].

However when co-expressed with p53 (tumor suppressor), it is localized along with p53 in the nucleus. NS3 unwinds both RNA and DNA substrates, although there is no DNA intermediate involved in HCV lifecycle. It couples unwinding of RNA strands (with extensive secondary structures) to Adenosine triphosphate (ATP) hydrolysis. A direct interaction between NS3 and NS5B occurs through the protease domain of NS3. RNA unwinding activity of NS3 helicase is modulated by this interaction with NS5B polymerase[68].

In addition to its role in viral polyprotein processing and HCV multiplication, another important function of NS3 involves antagonizing host innate-immune pathways. The induction of type I interferon (IFN) genes (Type I IFNs include several IFN-α subtypes and a single IFN-β subtype) is regulated at the step of transcription and is best understood for the IFN-β promoter. Innate immune defense mechanisms activated by alpha/beta INFs represent an essential first line of protection against viral infections. Retinoic acid inducible gene or RIG-I is a cytoplasmic RNA helicase [69].

NS3 undergoes other posttranslational modifications, such as phosphorylation and N-terminal acetylation [70]. The multifunctional roles played by NS3 in the lifecycle of HCV, makes it indispensable for virus multiplication. NS3 therefore represents a very promising target for anti-HCV therapy [27].

III.A.3.iii. HCV NS4A:

Nonstructural protein 4A (NS4A) is a cofactor for NS3 serine protease activity. The N-terminal portion of 4A is responsible for membrane association of the NS3-4A complex. NS4A also associates with other HCV proteins on the ER-derived Membranous webs, where viral replication complex is assembled. Interestingly, NS4A is localized not only on the ER, but also on mitochondria [71]. Thus, in addition to its essential role in HCV replication it is also involved in viral pathogenesis by affecting cellular functions. Further, a study reported altered intracellular distribution of mitochondria and its damage due to NS4A expression in Human hepatocyte -7 (Huh7) cells [72].

III.A.3.iv. HCV NS4B:

The HCV nonstructural protein 4B (NS4B) is a 27-KDa polypeptide. It is a highly hydrophobic integral ER-membrane protein [73]. It contains four trans membrane domains and is palmitoylated at two C-terminal cysteine residues. Palmitoylation of 4B protein facilitates oligomerization which appears to be essential for HCV replication [74].

It has an amphipathic helix at the N-terminal and C-terminal domains which are both associated with membranes [75]. Allelic variation in the NS4B sequence between closely related HCV isolates was found to drastically impact HCV replication in cell culture [76].

III.A.3.v. HCV NS5A:

The non-structural protein 5A (NS5A) has RNA-binding activity [77]. NS5A protein can be found in basally phosphorylated (56 kDa) and hyper phosphorylated (58 kDa) forms in cells. P58 form of NS5A is hyper phosphorylated at additional sites that remain unmodified in the p56 form by casein kinase I-alpha [78].

NS5A has an amphipathic alpha-helix at its amino terminus with which it is anchored to the ER membrane. NS5A is further divided into three-domains which are separated by linker regions. The crystal structure of the conserved domain I (DI) was solved recently and revealed a unique fold. DI has a zinc binding motif and also forms a nucleic acid-binding domain. NS5A specifically binds to the Guanine/ uracil nucleotides (G/U) rich sequences in the 3' ends of HCV genomic RNA [77].

Domains II (DII) and III (DIII) are more variable among HCV genotypes. Crystal structures for both DII and DIII are not available yet. Nuclear magnetic resonance (NMR) studies have shown DII of NS5A to be flexible and disordered. NS5A-DII contains the PKR and the HCV-NS5B binding domains. A role for NS5A-DIII in virus assembly was shown via phosphorylation of a serine residue at position 2433 by Casein kinase II (CKII)[79]. NS5A domain III is not required for RNA replication as sub-genomic replicon lacking DIII, replicate in Huh7 cells. NS5A also has the ability to inhibit IFN-gamma production [80]. A remarkable study with NS5A transgenic mice suggested that NS5A protein could impair both the innate and adaptive hepatic immune response [81].

III.A.3.vi. HCV NS5B:

The HCV nonstructural protein 5B (NS5B) is a RNA dependent RNA polymerase (RDRP) containing the GDD motif in its active site [82]. It belongs to a class of integral membrane proteins termed tail-anchored proteins. NS5B initiates synthesis of complementary negative-strand RNA using the HCV genome (positive polarity) as a template. Subsequently it generates positive-strand RNA from this negative strand RNA template. The NS5B crystal structure shows the typical fingers, palm and thumb sub-domains. NS5B is anchored to the ER-derived "membranous webs" via its C-terminal 21 amino-acid residues [83].

Studies with sub-genomic replicon revealed that the membrane association is indispensable for viral RNA replication [84]. In *vitro* NS5B cannot distinguish between natural and synthetic templates. NS5A can directly bind to NS5B and modulate its polymerase (poly A template/poly U primer) activity [85]. NS5A (at substoichometric levels) stimulates replication by NS5B on templates derived from the 3' end of the positive strand [86].

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III.B. The replication cycle

III.B.a. HCV lifecycle

Key steps in the life cycle of HCV include entry into the host cell, uncoating of the viral genome, and translation of viral proteins, viral genome replication, and the assembly and release of virions. All these events occur outside the nucleus of the host cell [87] fig (4, 5)

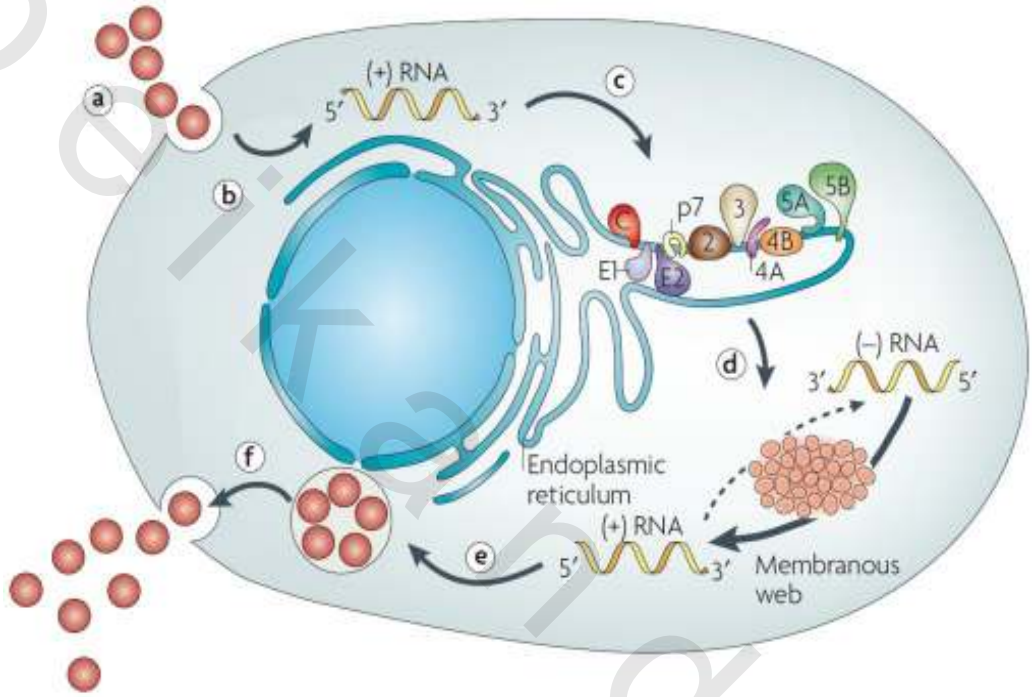

Figure (4). Schematic representation of the lifecycle of hepatitis C virus [88]. Virus binding and internalization (**a**); cytoplasmic release and uncoating (**b**); IRES mediated translation and polyprotein processing (**c**); RNA replication (**d**); packaging and assembly (**e**); virion maturation and release (**f**). The topology of HCV structural and non-structural proteins at the endoplasmic reticulum membrane is shown schematically. HCV RNA replication occurs in a specific membrane alteration, the membranous web. Note that IRES-mediated translation and polyprotein processing, as well as membranous web formation and RNA replication, which are illustrated here as separate steps for simplicity, might occur in a tightly coupled fashion. IRES, internal ribosome entry site. Adapted from [88].

III. B.a.i. Viral Entry:

Various factors attributed to the host cell seem necessary to enable HCV entry. The first factor that was identified as necessary for HCV entry was the tetraspanin, CD81. Although CD81 is expressed on many cell types and cannot explain HCV's liver tropism, However, HCV entry is strongly reduced in the presence of antibodies to CD81, or when CD81 expression is down regulated in hepatoma cells [87]. The human scavenger receptor class B type I (SR-BI) is thought to be an additional factor that might mediate entry of

HCV into cells. SR-BI is a physiological HDL receptor that mediates selective HDL-cholesterol uptake, but can also bind to other ligands, some of which affect HCV infectivity [89].

The components of HCV that are presumed to act as ligands for the receptors include the HCV envelope glycoproteins, E1 and E2, which have an essential role in entry of HCV into host cells [90].

III. B.a.ii. Uncoating and Translation:

After entry of the viral genome into the host-cell cytoplasm, the virus undergoes an uncoating process to expose the viral genome to host-cell machinery. The viral genome is then translated in preparation for viral replication as shown in figure (5) [90].

The 5' non translated region of the HCV genome contains an internal ribosome entry site (IRES) that permits ready access of the viral genome to the host translation machinery for viral-protein synthesis. IRES-mediated translation is a common mechanism used by many viruses to enable ongoing viral translation. This modality of translation is cap-independent enables viral translation to continue even after host cap-dependent translation has been shut down in response to viral infection. Translation of the positive-strand viral genome generates nonstructural and structural viral proteins, which are critical for viral replication and assembly of new virus particles, respectively [91].

Translation of the HCV genome produces a single ~3,000 amino-acid polyprotein which is processed by cellular and viral proteases into at least 10 different protein products. These products include the structural proteins, which form the viral particle (the virus core and the envelope proteins E1 and E2), and the nonstructural proteins P7, NS3, NS4A, NS4B, NS5A and NS5B [92].

After translation of the viral proteins that are necessary to establish the viral-replication machinery, viral RNA replication begins similar to other positive-strand RNA viruses, HCV is believed to replicate in association with intracellular membranes the positive-strand, viral RNA genome serves as a template for the synthesis of a single, negative-strand RNA. These two RNA strands remain base-paired, which results in the formation of a double-stranded RNA molecule that is copied multiple times by semi conservative replication to generate multiple progeny, positive-strand, viral RNA genomes. The double-stranded RNA intermediate is one of the pathogen-associated molecular patterns that is recognized by the innate immune system [93].

A key protein responsible for viral RNA synthesis is the HCV NS5B--the catalytic subunit of the replication complex, which has RNA-dependent RNA polymerase (RdRp) activity. Importantly, the HCV RdRp lacks a proofreading function and is therefore, highly error-prone. The lack of proofreading function results in the facile generation of genetic diversity, such that the virus population within an infected individual is best viewed as many different, but closely related, genomes, referred to as a quasispecies. This genetic diversity provides an ideal pool from among which the viral genome that is best adapted to a given antiviral intervention is selected, a concept that is clinically manifested as resistance to treatment [94].

III. B.a.iii. Viral Assembly and Release:

Secreted HCV particles have a characteristic low density, which suggests that the virus associates with lipoproteins for viral release. The association of HCV with lipoproteins might also protect the secreted virus from the host's immune response[95].

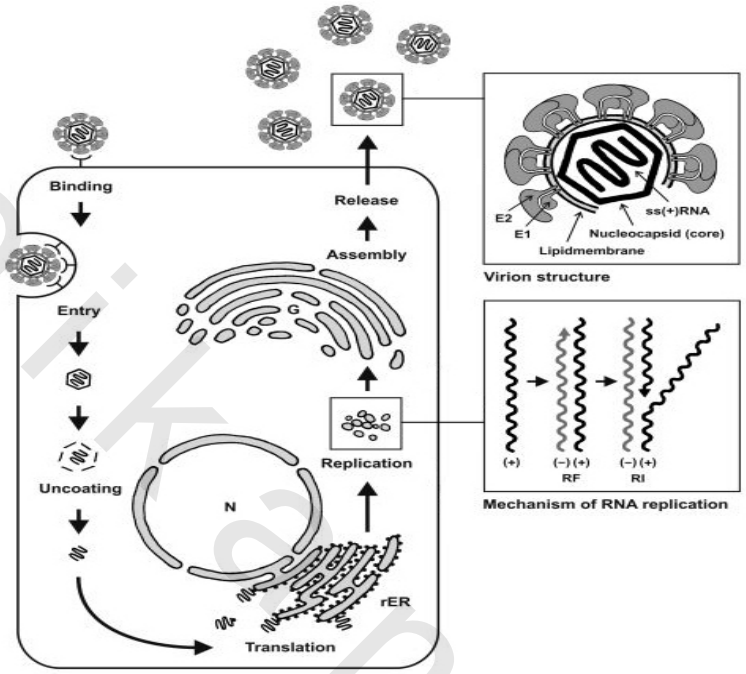

Figure (5). Hypothetical HCV replication cycle [90].

HCV particles bind to the host cell via a specific interaction between the HCV envelope glycoproteins and cellular receptors. Bound particles are probably internalized by receptor-mediated endocytosis. After the viral genome is liberated from the nucleocapsid and translated at the rough ER, NS4B induces the formation of membranous vesicles (referred to as membranous web). After genome amplification and HCV protein expression, progeny virions are assembled. The site of virus particle formation may take place at intracellular membranes derived from the ER or the Golgi compartment. Newly produced virus particles may leave the host cell by the constitutive secretory pathway. The upper right panel of the figure shows a schematic representation of an HCV particle. The lower right panel shows a model for the synthesis of negative strand (-) and positive strand (+) progeny RNA via a double stranded replicative form (RF) and a replicative intermediate [90].

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III.C. Genotypes:

Genomic heterogeneity and classification systems of HCV:

HCV is classified in the *Hepacivirus* genus within the *Flaviviridae* family [96] **,** more HCV has been classified into genotypes and subtypes. Initially, genotypes were considered to differ more than 20% at the nucleotide level and more than 15% at the amino acid level. However, phylogenetic analyses indicated that genotype 10 is closely related to genotype 3 and genotype 7, 8, 9 and 11 to genotype 6. Therefore, a new consensus nomenclature system was proposed to be used for HCV classification [97].

According to this system, HCV is classified into genotypes on the basis of < 70% similarity of nucleotide sequence and phylogenetic relationship. The more closely related HCV strains (75-80% sequence similarity) within some genotypes are designated subtypes. Genotypes are numbered in order of discovery and subtypes are assigned lowercase letters (Table 1). Nowadays, HCV is classified into 6 major genotypes and more than 90 subtypes. Sequence variation between the HCV genomes was on average 0.9%, indicative of the presence of HCV quasispecies in hepatitis C [98].

Table 1. Genotypes/subtypes that are presently defined in the database [98].

1	a, b, c, d, e, f, g, h, i, j, k, l, m
2	a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r
3	a, b, c, d, e, f, g, h, i, k, l
4	a, b, c, d, e, f, g, h, k, l, m, n, o, p, q, r, s, t
5	a
6	a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t

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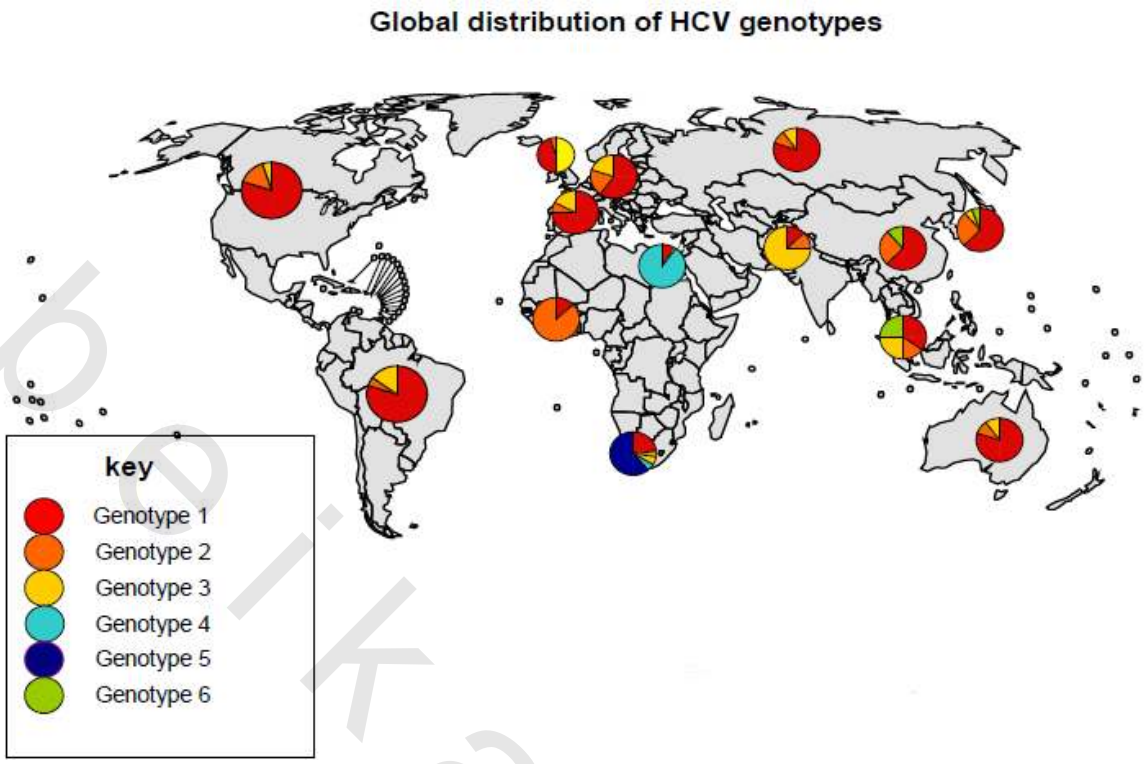

Figure (6). HCV genotypes prevalence. The boundaries and names shown and the designations on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement [99].

For example, in North America, genotype 1a predominates followed by 1b, 2a, 2b, and 3a. In Europe, genotype 1b is predominant followed by 2a, 2b, 2c, and 3a. Genotypes 4 and 5 are found almost exclusively in Africa. Most of the Egyptian sequences clustered with genotype 4, forming 5 distinct groups. The largest group clustered with subtype 4a. Genotype is clinically important in determining potential response to interferon-based therapy and the required duration of such therapy. Genotypes 1 and 4 are less responsive to interferon-based treatment than are the other genotypes (2, 3, 5 and 6) [100]. Duration of standard interferon-based therapy for genotypes 1 and 4 is 48 weeks [101], whereas treatment for genotypes 2 and 3 is completed in 24 weeks [102].

Infection with one genotype does not confer immunity against others, and concurrent infection with two strains is possible. In most of these cases, one of the strains removes the other from the host in a short time. This finding opens the door to replace strains non-responsive to medication with others easier to treat [103].

Responses can vary by genotype. Approximately 80% of hepatitis C patients in the United States have genotype 1, and genotype 4 is more common in the Middle East and Africa. Where, in genotypes 1, SVR is about 50% in patients with HCV genotype 1 given 48 weeks of treatment. In patients with HCV genotype 1, if treatment with pegylated interferon plus ribavirin does not produce a 2-log viral load reduction or complete

clearance of RNA (termed "early virological response") after 12 weeks the chance of treatment success is less than 1%[104], In genotypes 2 and 3 are SVR of 75% or better are seen in people with HCV genotypes 2 and 3 with 24 weeks of treatment [102]. Patients achieving HCV RNA below 1000 International unit per milliliters (IU/mL) by day 7 (i.e. just prior to the second dose of pegylated interferon) may be treated for as little as 12 weeks with retained sustained cure rates [105] and in genotype 4 are Sustained Virological Response (SVR) is about 65% in those with genotype 4 given 48 weeks of treatment [101].

IV. Treatment of HCV infection

IV.A. Initial Treatment:

The very first treatment shown to be effective against HCV involved systematic administration of INF-α. INFs are produced naturally by host's immune system in response to a viral infection. INF was actually discovered in 1957, during studies with influenza virus [106]. Through the years HCV treatment has improved with the development of modified pegylated interferon (Polyethylene glycol interferon (PEG-INF)), which is a stabilized version, has longer biological half-life[27].

Up to now, the standard of care (SOC) treatment consists of (pegylated) interferon-Alfa and ribavirin. However, depending on the viral genotype, treatment response rates differ significantly among infected patients. While up to 80% of the genotype 2 and 3 infected and 40–50% in genotype 1 patients can be cured, the response rate of genotype 4 in many clinical repots is showing SVR rates exceeding 60%[107]. There is no doughty that the high treatment cost presents a high economic burden in developing country like Egypt, necessitating a more meticulous research on predictors of (SOC) treatment response. Such as viral factors as viral load, genotype and host factors as steatosis, gender, liver cirrhosis and genetics as Interleukin-28B (IL28B) polymorphisms [108].

IV.B. Treatment Objectives and Outcomes:

The goal of therapy is to prevent complications and death from HCV infection. Because of the slow evolution of chronic HCV infection over several decades, it has been difficult to demonstrate that therapy prevents complications of liver disease. Accordingly, treatment responses are defined by a surrogate virological parameter rather than a clinical endpoint. Short-term outcomes can be measured biochemically (normalization of serum Alanine aminotransferase (ALT) levels), virologically (absence of HCV RNA from serum by a sensitive Polymerase chain reaction (PCR)-based assay), and histologically (< 2 point improvement in necro-inflammatory score with no worsening in fibrosis score) [109].

Several types of virological responses may occur, labelled according to their timing relative to treatment. The most important is the sustained virological response (SVR), defined as the absence of HCV RNA from serum by a sensitive PCR assay 24 weeks following discontinuation of therapy .This is generally regarded as a "virological cure," although liver cancer has been identified years later, especially if cirrhosis existed at the time of achieving an SVR[110].

Undetectable virus at the end of either a 24-week or 48-week course of therapy is referred to as an end-of treatment response (ETR). An ETR does not accurately predict that an SVR will be achieved but is necessary for it to occur [110].

A rapid virological response (RVR), defined as undetectable HCV RNA at week 4 of treatment, using a sensitive test with a lower limit of detection of 50 IU/ml, predicts a high likelihood of achieving an SVR [111].

An early virological response (EVR) is defined as a ≥ 2 log reduction or complete absence of serum HCV RNA at week 12 of therapy compared with the baseline level. Failure to achieve an EVR is the most accurate predictor of not achieving an SVR. Monitoring viral kinetics is thus useful for predicting whether or not an SVR is likely to develop. Virological breakthrough refers to the reappearance of HCV RNA while still on therapy, while virological relapse is the reappearance of HCV RNA in serum after treatment is discontinued and an ETR was documented. Persons who fail to suppress serum HCV RNA by at least 2 logs after 24 weeks of therapy are null responders, while those whose HCV RNA levels decrease by 2 logs IU/mL but never become undetectable are referred to as partial non responders [111].

Table 2. Treatment outcome classification according to generally accepted terminology [112].

Rapid Virological Response (RVR) = Negative HCV-RNA in week 4 of treatment
Early Virological Response (EVR) = HCV-RNA reduction > 2 log after 12 treatment weeks
Primary Non-Response (PNR) = less than 2 log decrease of viral titer after 12 weeks
End of Treatment Response (EoTR) = no detectable serum HCV-RNA at end of treatment
End of Treatment Non-Response = detectable serum HCV-RNA at end of treatment
Sustained Virological Response (SVR) = undetectable HCV-RNA 6 months after end of treatment
Relapse = detectable HCV-RNA in serum at any time after having achieved EoTR

IV.C. The Optimal Treatment of Chronic HCV:

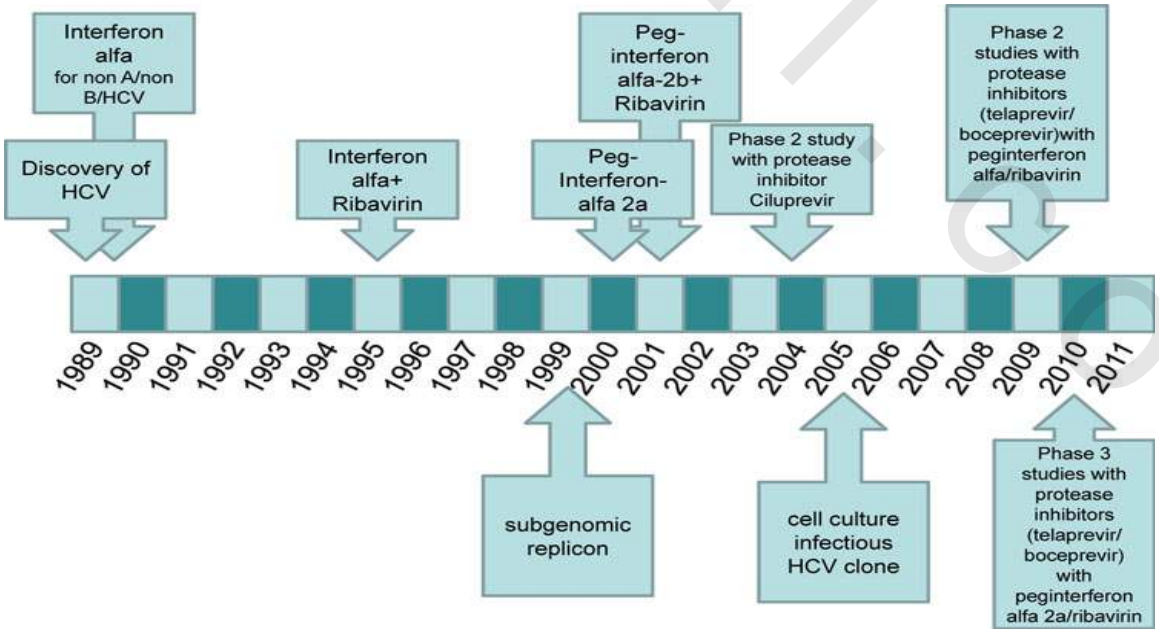

Figure (7). The development of HCV [113].

IV.C.i. Peg interferon Alfa and Ribavirin

The currently recommended therapy of chronic HCV infection is the combination of a pegylated interferon Alfa and ribavirin. The choice of this regimen was based upon the results of three pivotal, randomized, clinical trials that demonstrated the superiority of this combination treatment over standard interferon alfa and ribavirin, the optimal duration of therapy and the need for a different regimen for patients with genotype 1 and genotype 2 and 3 infections [114].

There are two licensed pegylated interferon's in the United States, peginterferon alfa-2b (Peg-Intron, Schering Plough Corp., Kenilworth, NJ), with a 12-kd linear polyethylene glycol (PEG) covalently linked to the standard interferon alfa-2b molecule, and peginterferon alfa-2a (Pegasys, Hoffmann-La Roche, Nutley, NJ) with a40-kd branched PEG covalently linked to the standard interferon alfa-2a molecule. The doses of these two forms of pegylated interferon differ The optimal dose of peginterferon alfa-2b, based on the original registration trial, is 1.5 µg/kg/week (microgram per kilogram every week) dosed according to body weight[115].

Although the dose of ribavirin used in the original registration trial was fixed at 800 mg daily, a subsequent community-based study of patients with genotype 1 infection demonstrated that weight-based ribavirin (800 mg for patients < 65 kg; 1,000 mg for patients weighing 65 to 85 kg; 1,200 mg for patients weighing 85 to 105 kg; and 1,400 mg for patients weighing > 105 kg to125 kg) was more effective[116].

Peg-interferon alfa-2a is administered at a fixed dose of 180 µg/week given subcutaneously together with ribavirin 1,000 to 1,200 milligram (mg) daily, 1,000 mg for those who weigh >75 kilogram (kg) and 1,200 mg for those who weigh >75 kg the optimal duration of treatment should be based on the viral genotype. Patients with genotype 1 should be treated for 48 weeks with peginterferon alfa-2a plus standard weight-based ribavirin, whereas patients with genotypes 2 and 3 could be treated with peginterferon alfa-2a plus low dose ribavirin (800 mg) for 24 weeks[117].

For patients with HCV genotype 4 infections, combination treatment with pegylated interferon plus weight-based ribavirin administered for 48 weeks appears to be the optimal regimen, as concluded in a meta-analysis of six randomized trials. While data from another randomized trial of treatment with combination peginterferon alfa-2b plus a fixed dose of ribavirin (10.6 mg/kg per day) has suggested that 36 weeks duration of therapy is sufficient provided an EVR is achieved [117].

IV.C.i.a. Interferon and its signaling pathway:

About 50 years ago, IFN was discovered by Isaacs and Lindenmann [118]. Currently, there are about 10 mammalian IFN species with many subspecies. IFN possesses antiviral activity and is categorized into three groups [119]. Type I IFNs include IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω and IFN-ν. All these interferon's interact with the interferon alpha/beta receptor (IFNAR) [120].Type II IFN involves only IFN-γ which interacts with a discrete receptor, the interferon gamma receptor (IFNGR) [121]. IFN-λ1, IFN-λ2 and IFN-λ3 are grouped in type III IFNs. These interferon are also known as IL29, IL28A and IL28B respectively. Type III IFNs, signal through IFN-λ receptor which possesses an IL-10R2 chain shared with the IL-10 receptor, and an exceptional IFN-λ chain [122].

IFNAR is the receptor for type I IFNs. It possesses two major subunits: IFNAR1, which binds tyrosine kinase 2 (TYK2) [123], and IFNAR2c which binds Janus kinase 1 (JAK1) [124]. Both TYK2 and JAK1 are members of the Jak family. After binding the receptor chains, TYK2 and JAK1 are stimulated and trans activated leading to the initiation of phosphorylation cascades involving all the members of the signaling pathway and also the activators of transcription such as signal transducer and activator of transcription STAT1, STAT2 and STAT3. STAT1, STAT2 and STAT3 are stimulated by type I IFNs in most of cells. STAT1 and STAT2 in combination with another transcription factor, interferon regulatory factor 9 (IRF9), form interferon stimulated gene factor 3 (ISGF3) which binds to the promoter region of interferon stimulated genes (ISGs) as shown in (Figure 8) [125].

The ISGs are a set of genes used for antiviral protection .Microarray analysis of human and murine cells treated with interferons revealed that there are more than 300 ISGs[126]. Most important of these proteins are the double-stranded RNA-dependent kinase "protein kinase RNA-regulated" (PRKR), the 2′–5′ oligoadenylate synthetases and the Mx proteins. These proteins are known to impede the growth of certain viruses. However, if these genes are knocked out from cells, they still retain their antiviral activities as there are many of other genes stimulated by interferon possessing antiviral activities [127].

Alternatively, STAT1 and STAT3 form homodimers or heterodimers which bind gamma activated sequence (GAS) elements. After binding, STAT proteins activate these genes to generate an antiviral state [128].Receptors and pathways involved in type III IFNs signaling diverge from those mediating type I IFNs signals. IFN-λ1-3 signal through the JAK/STAT signaling pathway using the IL28-R/IL-10R receptor complex that is mainly expressed on hepatocytes and epithelial cells [129] as opposed to IFNAR that are broadly expressed [125].

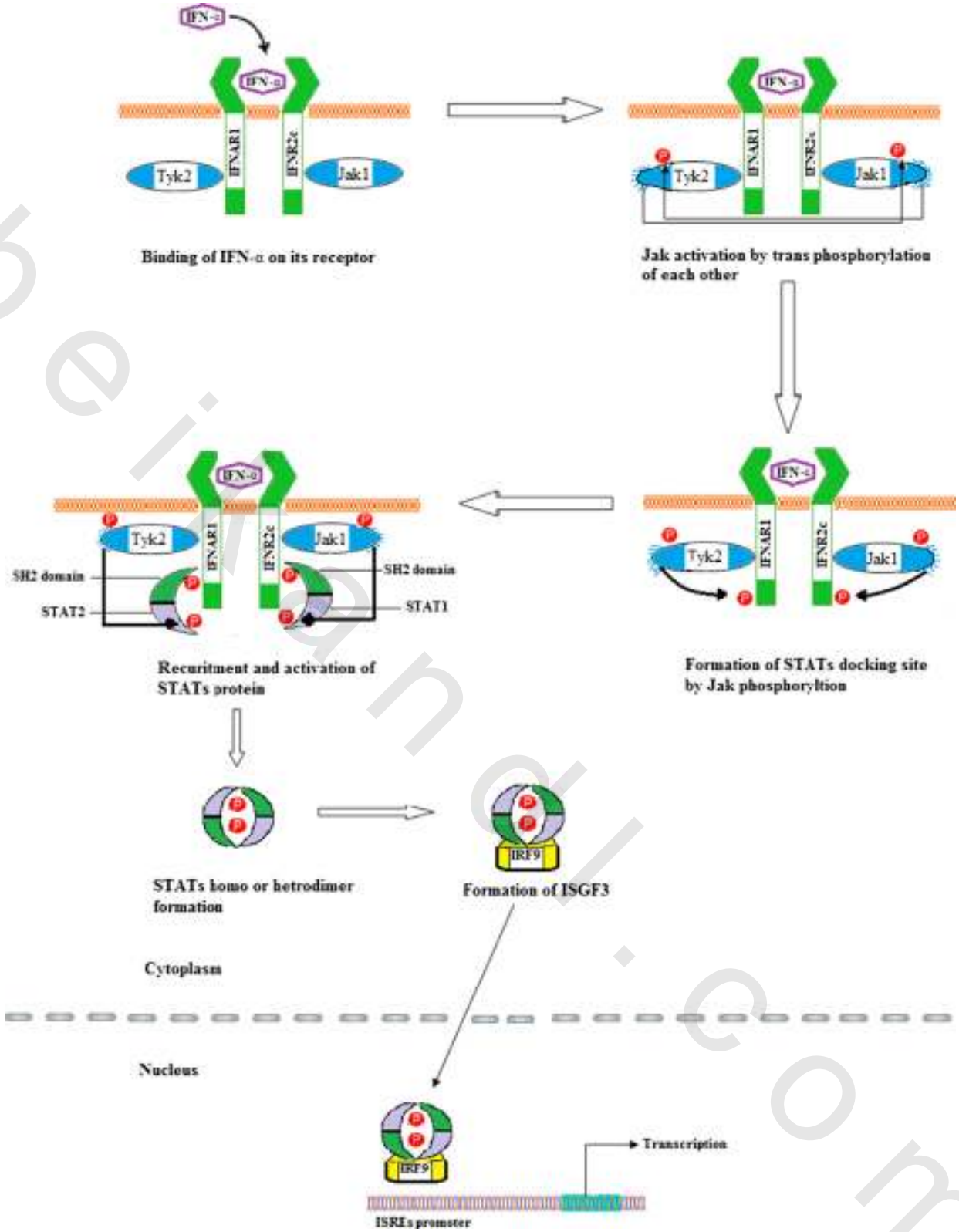

Fig. 8. Interferon signaling pathway [125].

IV. C.i.b. Ribavirin:

Ribavirin (1-b-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) inhibits HCV replication. It is a synthetic guanosine nucleoside analogue that inhibits inosine monophosphate dehydrogenase leading to the depletion of the guanosine pool [130].

It is also incorporated into the viral genome and induces error catastrophe by the HCV non-structural-5B (NS5B) polymerase [131]. In addition to these functions, it is thought to have immune-modulatory functions. It regulates macrophages, T helper cells and Th1/Th2 produced cytokines, enhances the expression of interferon stimulated genes (ISG) and also IFN-α and Myxovirus Resistance Gene A (MxA) expression [125].

IV. C.i.c. Side effects of HCV therapy:

Table 3. Side effects of HCV therapy (modified from [132].

Frequency of side effects	Interferon alpha	Ribavirin
>30%	- Influenza-like symptoms - Headache, Fatigue - Fever - Myalgia, Rigors - Thrombocytopenia - Induction of autoantibodies	- Hemolysis - Nausea - Abdominal pain
1-30%	- Anorexia - Diarrhea, Abdominal pain - Depression,Insomnia - Emotional lability, Irritability - Dermatological problems - Thyroid dysfunction - Leukocytopenia - Induction of autoimmune diseas	- Anemia - Nasal -congestion - Cough, - Laryngitis - Pruritus
<1%	- Polyneuropathy, Optic neuritis - Diabetes mellitus, Retinopathy - Paranoia or suicidal ideation - Cardiotoxicity, Seizures	Gout

Over the last years, HCV associated end-stage liver disease has become the most frequent cause for liver transplantation in Switzerland, Europe and the US [133].

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V. Factors determining the response to therapy

V. A. Viral Factors:

V. A.i. Genotype:

HCV sequences which varied up to 33-36% were classified as genotypes; sequences which varied up to 20 – 27% were classified as subtypes while genetic variants up to 12% within individual isolates were classified as quasi species. The HCV genotype is acknowledged as the most significant independent response marker of interferon therapy [134].

HCV genotypes 2 and 3 are more responsive to interferon therapy than any other HCV genotype [135]. Although HCV genotyping is an important predictor for the treatment response, no patient should be left without therapy on the basis of the genotype alone [136] because the predictive value of HCV genotyping for interferon based therapy is only 55% [125].

V. A.ii. 5′UTR

The important role of 5′UTR (Untranslated Region) in translation of HCV polyprotein demands the conservation of this region. There are four highly conserved structural domains in 5′UTR which are numbered I to IV; all of these domains interact with the host factors and are crucial for HCV polyprotein synthesis[137]. Domain III of 5′UTR binds eukaryotic initiation factor 3 (eIF3) by means of stem-loop 3b. It has been suggested that insertions in domain 3 of 5′UTR causes altered Watson-Crick base pairing leading to decreased RNA stability and binding affinity to ribosomal proteins. These mutations were more common in SVR than in breakthrough (BT) patients. Thus, mutations within the domain III of 5′UTR are important for the treatment response in HCV infection [138].

V. A.iii. Variations of core, p7, NS2, NS3 and NS5A:

Inter-patient genetic variations within the genotype 1 HCV also exert an important influence on the treatment response. Although sequencing of the HCV genome has not shown any specific amino acid sequences that affect the treatment outcome, the high levels of genetic variations, mostly in the core, p7, NS2, NS3 and NS5A genes, are linked with SVR [139].

Recent data from the Chinese population have also shown that increased genetic variations in the p7, NS2 and NS3 genes of HCV genotype 1b were linked with SVR to the standard treatment [140]. Thus, there is more genetic diversity in HCV SVR patients than in NR patients receiving the standard treatment.

Analyses of different HCV sequences identified many specific regions of core, p7, NS2, NS3 and NS5A, which were significantly associated with the ultimate treatment outcome. Among these regions, the most important region was the interferon sensitivity determining region (ISDR) that is located in HCV NS5A [141].

V.B. Host Factors:

The treatment response to HCV infection not only depends on the viral factors but also on the host factors. Male sex, older age, insulin resistance, diabetes, African or American ethnicity, cirrhosis, steatosis, and weight in terms of body mass index (BMI) are all factors linked to poor response to PEG-IFN plus RBV treatment [142]. Other infections such as Human Immunodeficiency Virus (HIV), HBV and practices such as alcohol intake and drug use are also responsible for low SVR rates [143].

V.B.1.Patient characteristics:

V.B.1. i. Age:

Patient age is an important factor linked to the treatment response to HCV infection. In general, it is assumed that younger individuals below forty respond better to interferon therapy [144] than older ones. The obvious justification for this association is that aged patients are more likely to have other liver diseases, such as fibrosis and cirrhosis. Moreover, in older age there are more imbalances of cellular, humeral, and innate immunity [145].

V.B.1. ii. Race:

Race is another important host factor that is linked with the treatment outcome[146]. There is increasing evidence that African-American patients respond poorly to interferon therapy compared to non-African-Americans. The range of sustained response for African-Americans was 19%-28% while those for non-African-Americans were 39- 52%. Moreover, the viral breakthroughs were also more frequent in African-Americans [147]. The mechanisms behind these observed differences in the treatment response are not properly cleared. It may be due to higher body weight and the HCV genotype 1 prevalence in African-Americans [148].

Another important reason for this low treatment response among the African-American population is the low prevalence of IL28B polymorphism (rs12979860). The prevalence of this SNP among the African-American is only 16% with SVR rate of 47% while among the Caucasians its prevalence is 39% with SVR rate of 81% [149].

V.B.1.iii. Sex:

Initially it was shown that there were significant differences in SVR rate with the female. Female sex is positively associated with SVR [150]. However, large prospective studies showed that there was no effect of sex in achieving SVR [151].

V.B.1. iv. Obesity:

It has been shown that a body mass index (BMI) > 25 kg/m^2 was linked with fibrosis [152]. Approximately, 30% of HCV patients are obese and they respond poorly to interferon therapy[153]. The poor treatment response in these patients is mostly attributed to altered metabolism due to cytokine production by adipocytes. Moreover, there is also a poor absorption of interferon in obese patients [154].

Moreover, obesity is associated with decreased number and down regulation of insulin receptors and impairment of post-receptor signaling. Overflow of free fatty acids

(FFAs) from adipose tissue interferes with intrahepatic insulin signaling pathway *via* increased levels of pro-inflammatory cytokines such as Tumor necrosis factor (TNF-α)[155], and proteasomal degradation of the insulin receptor substrates (IRS) 1 and 2 [156]. (Figure.9)

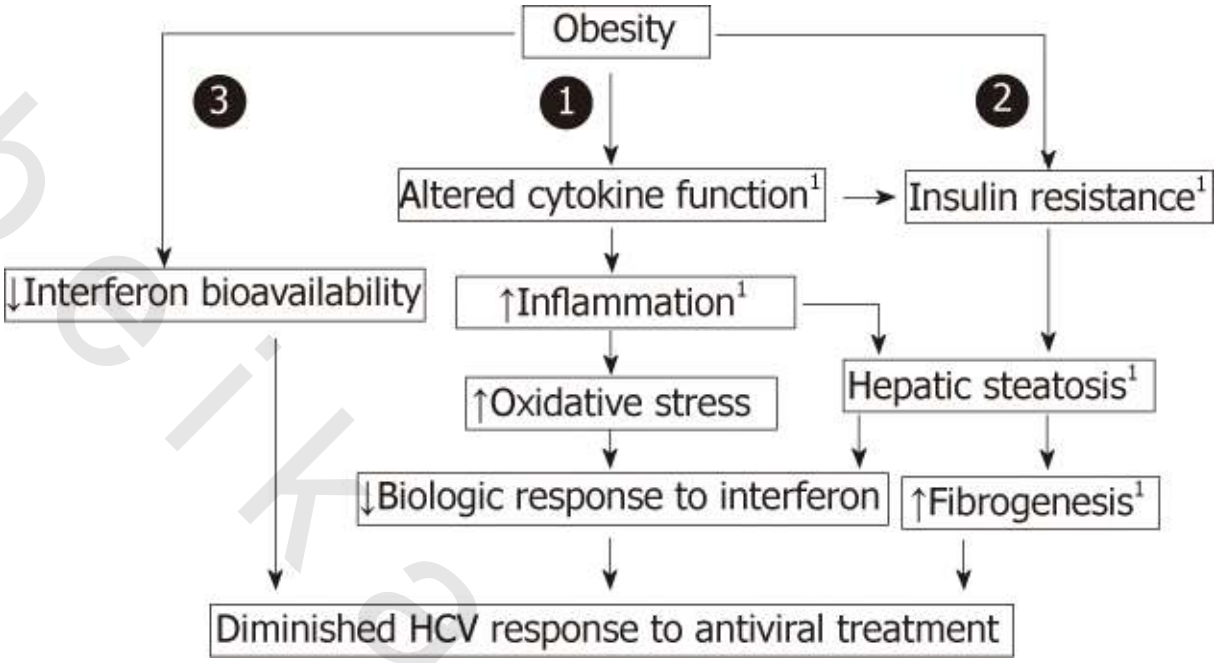

Figure (9). Obesity is associated with insulin resistance and decreased antiviral efficacy. Indicates potential for coexistent viral potentiation HCV (Hepatitis C virus)[157].

On the contrary, a recent large study showed that there is no significant effect of BMI on the treatment outcome [158]. However, weight loss plays an important role in HCV treatment because it down regulates liver enzymes and the progression of fibrosis [159].

V.B.1.v. Hepatic steatosis

Hepatic steatosis accelerates disease progression in HCV patients [160]. There is an involvement of both host and viral factors in steatosis development .Recently owing to the advances in *invitro* model for HCV propagation, it has been demonstrated that lipid factors are linked to HCV RNA replication [161]. The lipid rafts, scaffold for HCV RNA replication, is made mainly by cholesterol and sphingolipid , [162] and lipid droplet, an organelle for storage of neutral lipids is critical for producing infectious viruses[163].

Different HCV genotypes are shown to have different associations with hepatic steatosis. In patients infected with genotype 3, steatosis is mostly virus-induced and often severe, correlates with intrahepatic viral load, and resolve after successful antiviral therapy [164]. In contrast, in patients infected with genotype 1, 4 steatosis is mainly associated with host metabolic factors and correlates with body mass index (BMI) and central adiposity [165].

V.B.1. vi. Insulin resistance (IR):

Multiple studies have shown that HCV infection is also linked with insulin resistance[166]. The risk of developing diabetes mellitus is increased up to 11 times in patients with chronic HCV infection [167].Interferon therapy is also affected by insulin resistance in chronic HCV patients. Insulin resistance causes up-regulation of suppressor of

cytokine signaling 3 (SOCS3) which hinders interferon-mediated signaling pathways [168] (Figure10).

HCV infection can be considered not only as viral disease but also as a special type of metabolic disease. Chronic hepatitis C (CHC) interacts with lipid metabolism leading to steatosis, impairs glucose metabolism leading to insulin resistance (IR) and type 2 diabetes mellitus (T2DM), and is associated with an increased risk carotid atherosclerosis [169]. Moreover recent clinical data suggest that HCV associated IR is genotype dependent (1-4)[170].

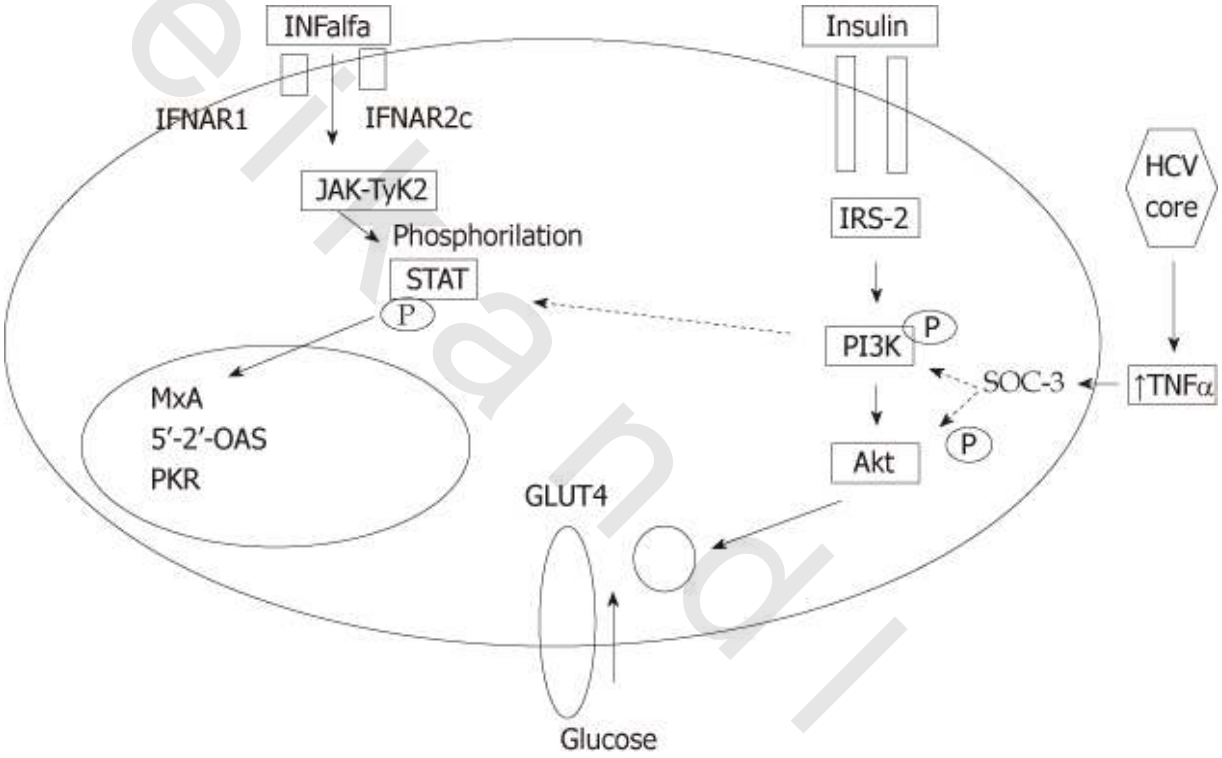

Figure (10). Interaction between hepatitis C virus core, insulin- and interferon-α signaling pathways continuous lines represent activation. Dotted lines represent inhibition. Hepatitis C virus core protein induces expression of tumor necrosis α (TNF-α), which in turn activates suppressor of cytokines-3 (SOCS-3). Activation of SOCs-3 leads to proteasomal degradation of insulin receptor substrate and inactivates phosphatidyl-inositol-3-kinase (PI3K), leading to inhibition of translocation of glucose transferase (GLUT-4) to cell membrane, blocking intracellular glucose entry, with subsequent hyperglycemia, hyper insulinemia and peripheral insulin resistance. Activation of SOCS-3 also leads leading to inhibition of Tyr-phosphorylation of signal transducers and activators of transcription 1 leading to impaired TNF-α signaling. HCV: Hepatitis C virus; IFNAR2c: Interferon receptor chain 2; IRS2: Insulin receptor substrate 2; JAK: Janus kinase; TYK2: Tyrosine kinase 2; STAT: Signal transducer and activator of transcription[157].

V.B.2. Single Nucleotide polymorphisms (SNPs) of host genes:

V.B.2. Interleukin 28B (IL-28B) polymorphism

The deoxyribonucleic acids (DNA) sequence polymorphisms are defined as the DNA sequence variations present at frequency greater than 1% of the human population. The most common are Single Nucleotide polymorphisms (SNPs). Other sequence polymorphisms include deletions and insertions of one or more nucleotides, rearrangements and repeating sequences which may be short tandemly repeated motifs of one to six nucleotides (microsatellites) or longer repetitions (minisatellites) [171].

Single Nucleotide Polymorphisms (SNPs) are mutations that occur at genome position at which one of the four possible nucleotides in the DNA sequence is substitute by another, resulting in two distinct nucleotide residues (alleles) that each appear in significant portion in human population (i.e. a minor or mutant allele frequency greater than 1% of the human population) [172]. There are some estimated 14 million SNPs [173] in the human genome that occur at a frequency of approximately one in 1,200-1,500 Base Pair (bp) [174]. SNP can affect protein function by changing the amino acid sequences (non-synonymous SNP) or by perturbing their regulation (affecting promoter activity [175], splicing process [176], as well as DNA and pre-messenger ribonucleic acids (m RNA) stability and translation by altering other polyadenylation or by altering other protein/m RNA regulatory interaction [177].

The majority of DNA sequence polymorphisms are of no functional importance although some polymorphisms can cause translational modification in structure of the resulting peptide [178]. Such non-synonymous or coding polymorphisms have classically been implicated in monogenic Mendelian disorders (i.e. disorders that are controlled by single locus) with their consequences typically predictable and amenable to testing. In contrast, regulatory polymorphisms occurring outside exonic (coding) regions and those in exonic regions but encoding for the untranslated regions of m RNA have long been assumed to be important modulators of gene expression and evolutionary change [179], but there is now growing evidence confirming that assumption [180].

Regulatory polymorphisms can be classified into two groups [171]; the *cis*-acting polymorphism which is typically present in or near the locus of the gene that it regulates. This may arise, for example, through the sequence change occurring in regulates. Alternatively, a *trans*-acting polymorphism occurs when a polymorphism in one distant gene is affect expression of another gene. There is evidence from differences in strains of mice and yeast that *trans*-acting loci were responsible for much of the observed differences in expression [181]. In the context of complex disease susceptibility studies have focused more on the role of *cis*-acting polymorphism than that of the *trans*-acting polymorphism [171].

Short (20-23 nucleotides) single-stranded non protein-coding endogenous RNA molecules termed microRNAs (miRNAs) have been demonstrated to regulate expression of a large proportion of genes [182] through binding to their target m RNAs in the 3′-UTR, using a partial base-pairing mechanism. This binding would result in either inhibited translation or even induction of target m RNA degradation depending upon, the overall degree of complementarity of the binding sites [183]. In order for a mi-RNA to give rise to

functional consequences, the 7-8 nucleotides of its 5′ end (generally referred to as the " seed" region) must have exact complementarity to the its target m-RNA binding site [184].

Importantly, SNPs which when transcribed create, destroy, or modify miRNA binding sites in the 3′-UTR of m-RNA are called (miRSNPs) and they represent a specific class of *cis*-acting regulatory polymorphisms that may lead to the dys-regulation posttranscriptional gene expression [182].

V.B.2.b. Interferon-λ:

The IFN-λ family was discovered in 2003. The three members of this family, IFN-λ 1, 2, 3, (equivalent to IL-29, 28A, 28B) show a high degree of homology to each other i.e. more than 80%, but very low sequence homology to both IFN-α (15-19% identity, 31-33% similarity) and IL-10 (11-13% identity, 22-23% similarity). Despite this minimal homology, IFN-λ like IL-10 gene is composed of five to six exons [185]. IFN-λ is produced by many immune cells, neuronal cells, alveolar epithelial cells, hepatocytes, and a variety of cell lines [186].

However, the primary sources of IFN-λ are dendritic cells (DCs) [187]. Similar to IFN-α, these cytokines are mainly produced in response to viral infection or by activation of Toll-like receptors (TLRs) [188]. IFN-λ showed antiviral activity against many viruses such as Encephalomyocarditis virus (EMCV), vesicular stomatitis virus, cytomegalovirus, herpes simplex virus 1, influenza A virus, HIV, HBV, and HCV [189]. These studies revealed that IFN-λ mainly inhibits viral replication but also has immune-modulatory functions. It modulates both the maturation and differentiation of immune cells [190].

In short, these cytokines have an important role in regulation and development of the adaptive immune response against viruses. SNPs within chromosome 19, in the vicinity of the IL29, IL28A, and IL28B genes, are importantly associated with the treatment response of HCV infection. Three SNPs related to these genes, rs8099917, rs12980275, and rs12979860, are very important. Previous studies have shown that these variants are significant predictors of the treatment response [191].

The favorable genotypes significantly predicting higher SVR rates are CC rs12979860 irrespective of the race [192], AArs12980275, and TTrs8099917. Nevertheless, the association between IFN-λ production and SNPs in close proximity of IL28 remains indistinct [193].

IFN-λ-based drugs are possible candidates for treating HCV infection, and are presently being evaluated in clinical trials. The adverse effects of IFN-λ are less marked than all of IFNs α and β, regarding bone marrow suppression, probably because fewer cells carry the receptor, thus permitting a more targeted therapy [194].

Genome-wide association studies (GWAS) reported the association between rs12979860 a Sustained Virological Response (SVR) in patients with genotype 1 and treated with PEG-IFN-α2a or PEG-IFN-α2b. An approximate two fold change in response to treatment was observed in patients with the SNP rs12979860 CC genotype compared with the TT genotype. The reported Odds Ratio (OR) was 7.3 in European-Americans, 7.1 in African-Americans and 5.6 in Hispanics .The SNP rs12979860 is located ~3 kbp upstream the IL28B gene and resulted the strongest predictor of SVR, compared to

baseline fibrosis or baseline viral load. Indeed, the actual causative variants have not been yet identified, although targeted sequencing of the IL28B region revealed two possible candidates: a non-synonymous substitution (rs8103142, K70A) and a variant in the promoter region of IL28B (rs28416813)[195]. Recently, gene expression has been linked to IL28B genotypes, thus providing a direct link between genotype and function. Indeed, lower pre-treatment intrahepatic expression levels of ISGs have been reported for the genotypes of rs8099917 and rs12979860 associated with SVR [196].

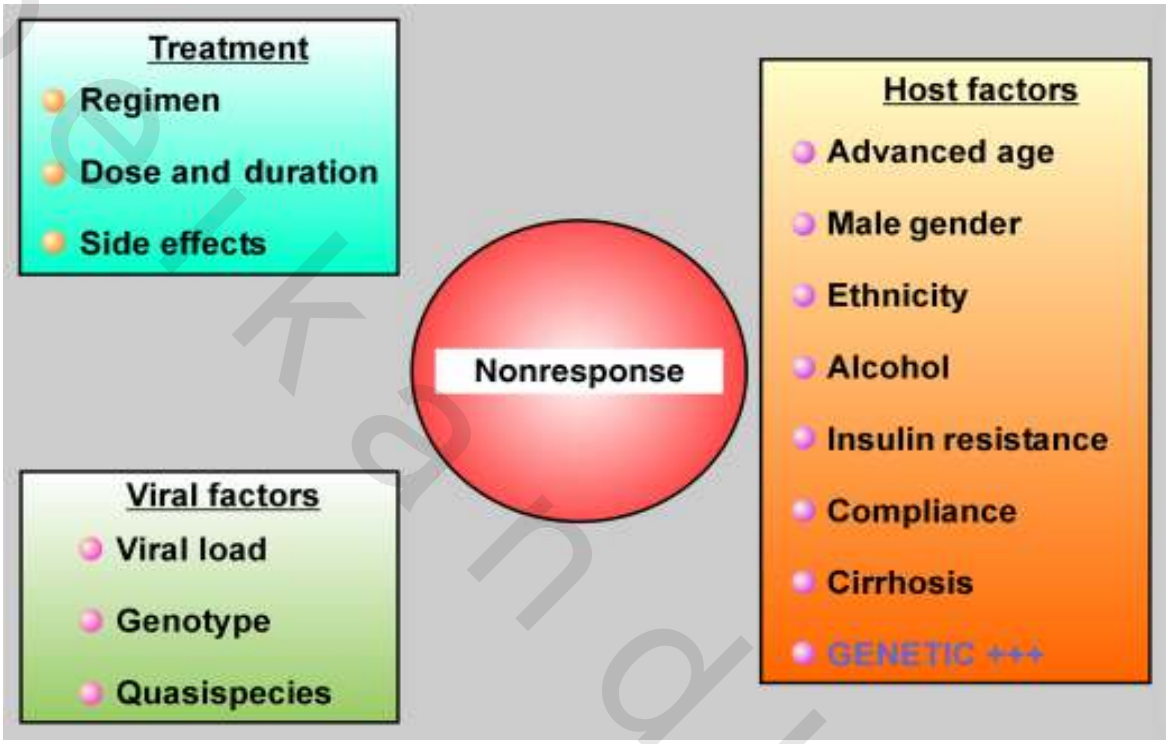

Figure (11). Factors associated to non-response to pegylated interferon plus ribavirin treatment [197].

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