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**An insight in the diagnosis and pat****hogenesis of hepatitis C virus infection**

Irshad M *et al*. Diagnosis and pathogenesis of HCV

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**Abstract**

The present review focuses on the findings in the area of diagnosis and pathogenesis of hepatitis C virus (HCV) infection in last few decades. The informations based on published literature give an update on these two aspects of HCV. HCV infection, previously called as blood transmitted non-A, non-B infection, is prevalent globally and poses a serious public health problem of the world. The diagnosis of HCV infections has been evolved from serodetection of non-specific and low avidity anti-HCV antibodies to detection of viral nucleic acid in serum using PCR technique. The current PCR assays detect viral nucleic acid with high accuracy and exact copy number of viral particles. Moreover, multiplex assays using real time PCR are available for identification of HCV-genotypes and their isotypes. In contrast to previous methods, the newly developed assays are not only fast and economic but also resolve the problem of window period as well as differentiate the present from past infection. HCV is a non-cytopathic virus and so, its pathogenesis is regulated by host immunity and metabolic changes including oxidative stress, insulin resistance and hepatic steatosis *etc*. Both innate and adaptive immunity play important role in HCV pathogenesis. The cytotoxic lymphocytes demonstrate a crucial activity during viral eradication or its persistence and are influenced by viral proteins, HCV-quasispecies and several metabolic factors regulating liver metabolism. HCV pathogenesis is a very complex phenomenon and needs more studies to extricate its other aspects.

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**Key words:** hepatitis C virus; diagnosis; pathogenesis; immunity; steatosis

**Core tip:** This article focuses on most important aspect of diagnosis and pathogenesis of hepatitis C virus infections. Both these aspects are important for the attempts being made in the direction of eradicating this virus from its endemic nature and inflicting world population facing formidable form of liver diseases.

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**INTRODUCTION**

Hepatitis C virus (HCV) was first characterized by Choo *et al*[1] and Kuo G *et al*[2] in 1989. It was soon identified as the main causative agent of previously called post transfusion non-A, non-B hepatitis virus. HCV has been found to be an important cause of liver diseases and remains a major public health problem of the world. According to World Health Organization, nearly 3% of the world population has been infected with HCV. And so, more than 170 million people are chronic carriers of HCV and at high risk of developing liver cirrhosis and/or hepatocellular carcinoma (HCC). Three to 4%of the chronically infected individuals develop fatal HCC. Now a days HCC caused by HCV infection is considered a prominent indication for liver transplantation[3-5].

HCV was the leadingcause of post-transfusion and community-acquired non-A, non-Bhepatitis until the characterization of virus in 1989 and introduction of blood screening in 1990.The institution of blood screening for HCV has markedly reducedits incidence. However, it still remains a significant problemin intravenous drug abusers. HCV infection is the mostcommon cause for liver transplantation in adults. HCV and HIV-1 frequently co-infect humans and ithas been estimated that as high as 18% of HIV-infected personsare also infected with HCV[4].

 HCV is an enveloped RNA virus and belongs to the genus Hepacivirus of the family flaviviridae. HCV genome consists of 9.6-kb single-stranded RNA of positive polarity and a single open reading frame of 9033-9099 nucleotides flanked by a conserved 5` and 3` noncoding region (NCR) at the ends. Its genome codes for a long polyprotein of approximately 3000 amino acids[6] which is processed co-translationally and post-translationally to yield structural proteins (core, envelope E1, and E2) and non-structural (ns) proteins (NS1/p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B)[7].The envelope proteins (E1 and E2) are the outer surface proteins of the viral particles and play important role in virus entry inside the host cell. NS5B is a variable region of HCV genome and codes for a RNA dependent RNA polymerase (RdRp).

RNA polymerase lacks proof reading activity and so, this may alter the detection, sensitivity to anti-viral activity of interferon and pathogenicity of virus (Figure 1) [8].

Like several other viruses, the RNA of virus has a high degree of heterogeneity[5] that varies 30%-35% among different genotypes. Based on previous studies six major genotypes and more than 120 subtypes of HCV have been characterized till date[9]. These HCV genotypes have distinct geographic distribution with genotype 1 and 2 frequently occurring worldwide[10]. In India Genotype 3 is reported to be the most prevalent, followed by genotype 1[11,12]. Different genotypes of HCV have important epidemiological implications. Despite nucleotide sequence divergence between genotypes, they remain quite similar in their transmission pattern, persistence and disease development[13]. Although genetic variation is attributed to several factors, however, two major theories *i.e.*, Darwinian and Neutral evolution theories have been reported as the prominent theories in causing genetic diversity in HCV[13]. The nucleotide sequence variability is distributed throughout the viral genome. Regions encoding envelope proteins (E1, E2) and NS-1 are the most variable, whereas the 5` NCR is the most conserved region.

HCV patients show a poor response to antiviral therapy based on the combination of pegylated interferon (IFN)-α and ribavirin. Only 40%-50% of patients infected with HCV genotype-1 and 80% of those infected with genotype-2 or 3 achieve a sustained virological response (SVR) with this regimen[14]. The recent use of direct acting anti-viral (DAA) molecules, that are active on HCV, during treatment has led to substantial improvement in SVR rates in HCV genotype-1 infected patients. However, it may lead to selection of resistant virus if DAA used alone[15]. Moreover, there is a high relapse rate of HCV infection after discontinuation of therapy. Recently, host genetic factors including human leukocyte antigen (HLA) and cytokine gene have been implicated in HCV infection or persistence[16]. Genetic polymorphism of cytokine genes including *IFN-γ*, *TNF-α*, *IL-10*, *IL-20* and *SNPs* in the promoter region of osteopontin gene, have been found crucial in determining the therapeutic outcome of HCV infection[17]. Therefore, every effort is being made to understand the pathogenesis of HCV infection so as to create a therapeutic model for an effective treatment against HCV. Although recent reports describe the development of *in vitro* replication systems leading to the production of infectious viral particles[18,19], there is currently no cell culture model suitable for synthesizing vaccines based on killed or attenuated virus. All efforts focus on sub-unit vaccines, composed of one or several antigens, either in the form of recombinant proteins, synthetic peptides or vectored vaccines. The earliest vaccine developed for HCV was that by the Chiron group[20] However, there was very little progress noted in this direction in subsequent years.

Present article reviews on few major aspects of HCV infection including the diagnosis and pathogenesis of HCV infection. Both these aspects have strong association with therapy and thus, explore possibility to further develop therapeutic model after availability of newer means of accurate diagnosis and better understanding of its pathogenesis. This attempt may update the readers about the informations available on these two aspects till date.

**DIAGNOSIS OF HCV INFECTION**

During HCV infection, every attempt is made to diagnose and differentiate acute from chronic hepatitis C infection. Acute HCV infection is typically mild. It is often not diagnosed, and the infection may be recognized only when it becomes chronic[21]. The diagnostic tests including presence of anti-HCV antibodies in serum cannot differentiate between acute and chronic HCV infection because anti-HCV IgM used as marker of acute infection occurs variably in acute infectious disease and is also detected at high rates in patients with chronic HCV infection[22,23]. While going in the history of diagnostic procedures of hepatitis C virus infection in laboratories, they are based on the detection of anti-HCV antibodies against recombinant HCV proteins using enzyme immunoassay (EIA) and Chemiluminescence immunoassay. Non-structural and recombinant antigens were used in these assays. Four different generations of anti-HCV test kits have been developed to date. The first generation EIA detected antibodies against the nonstructural proteins (NS4) with recombinant antigen c100-3. Subsequently, the second generation assay was developed and this included antigens from the core region (c22-3), the NS3 region (c33c) and a part of c100-3 (5-1-1) from the NS4 region. Later on, the third-generation EIA included an additional antigen from the NS5 region and a reconfiguration of the core and NS3 antigens. However, all these anti-HCV assays had the disadvantages of giving high false positive results and having lack of sensitivity to detect antibodies during window period. Also, these antibody-based assays could not distinguish between acute, past and chronic infections. It was followed by the development of supplementary tests involving recombinant immunoblot assay (RIBA) that were commercialized. This assay contained recombinant antigen (c33c, NS5) and synthetic peptides (5-1-1, c100 and c22). Similarly, few other commercial assays, termed as third generation line immunoassay incorporated HCV antigens from core region, E2 hypervariable region, NS3 region, NS4A, NS4B and NS5A region. All these recombinant immunoblot assays were used as supplementary tests of anti-HCV assays. Like EIA, the RIBA assays had the disadvantages such as difficulty in performance and high percentage of indeterminate results. Therefore, these are no more used in diagnostic laboratories. Recently, fourth generation anti-HCV assays incorporating additional nonstructural proteins are being used as screening test. [24] These kits for anti-HCV detection target different HCV antigens and detect more than five primary antibodies to ensure the specificity and sensitivity of detection kit.

Anti-C22c and anti-C33c may be the first HCV antibodies to appear during the acute phase of the disease, which is defined by elevated alanine aminotransferase (ALT) levels and/or clinical symptoms[25]. Anti-NS5 appears somewhat later, while anti-C100-3 is the last antibody to be detected in acute self-limited HCV infection. The diagnosis and differentiation of acute from chronic HCV infection poses another problem also. Patients chronically infected with one HCV-genotype develop acute hepatitis on infection with another genotype. Multiple episodes of acute hepatitis were observed in polytransfused thalassemic children reinfected with different HCV genotypes[26,27]. Therefore, discrimination between acute and chronic infection in the same patient sometimes becomes very difficult. HCV RNA in the serum or liver appears to be the earliest detectable marker of acute HCV infection, preceding the appearance of anti-HCV by several weeks[25]. HCV viremia may persist despite the normalization of serum ALT levels. And so, use of ALT levels is the diagnosis of HCV does not help much. However, HCV RNA in serum usually lasts for fewer than 4 mo in patients with acute self-limited HCV infection. The average time from transfusion to sero-conversion is approximately 11 to 12 wk with EIA-1 (Enzyme immunoassay-1) and 7 to 8 wk with EIA-2 (Enzyme immunoassay-2). Now attempts are being made to develop EIA assays to differentiate HCV sub-types also[28].Patients with post transfusion chronic non-A, non-B hepatitis develop anti-HCV antibodies in majority of cases. Anti-HCV antibodies are not neutralizing, especially with HCV envelope proteins E1 and E2[29]. High levels of anti-C100-3 were correlated with high titers of circulating HCV in chimpanzees[30]. Therefore, the development and persistence of diagnostic antibodies to HCV seem to reflect concomitant virus replication and consequently a high potential for infectivity.

HCV RNA is frequently detected in patients with chronic hepatitis C and anti-HCV antibodies carrying patients. One of study in Hong Kong reported 83% anti-HCV positive patients to be viremic when HCV RNA was tested by PCR with two different sets of primers for noncoding regions[27]. Similarly, in another study 98 of 100 patients with chronic non-A, non-B liver disease were positive for antibodies by EIA-2, but all 100 patients were positive for HCV RNA by PCR. With the currently available EIA systems, chronic HCV infection can readily be identified in most patients. Measurement of HCV RNA by PCR does not substantially increase the numbers of patients found to have chronic HCV infection[31].After introduction and wider use of real time PCR, now it has been easy to diagnose and monitor the progress of HCV viremia in very short time period[32].Not only this, the use of multiplex PCR by real time is another advancement in the direction of detecting possible hepatitis viral co-infections in single attempt analysis[33].

Based on published informations about various aspects of HCV infection including the currently available diagnostic assays and therapeutic regimen, American Association for the Study of Liver Diseases and Centers for Disease Control and Prevention, United States have approved a document as “practice guidelines” for its use in the diagnosis and treatment of HCV infection. This is an important document and describes details of guidelines to be followed for laboratory diagnosis of acute/chronic HCV infections[34].

**PATHOGENESIS OF HCV INFECTION**

HCV is a non-cytopathic virus[35] that enters the liver cell and undergoes replication simultaneous by causing cell necrosis by several mechanisms including immune mediated cytolysis in addition to various other phenomenon like hepatic steatosis, oxidative stress and insulin resistance. The proteins/peptides encoded by different sub-genomic regions of HCV genome and their quasispecies influence above mechanism and thus, show significant role in HCV pathogenesis and disease causation. Following is the brief description of HCV pathogenesis in the light of all these factors (Figure 2).

***Viral entry***

HCV is a blood-transmitted virus that reaches liver via circulation. Entry of HCV isolates requires at least 4 host-derived factors including scavenger receptor class B type I, Occludin, Claudin-I (CLDNI) and CD81. In addition, CLDN6 and CLDN9 have been shown to substitute for CLDN1 as HCV entry factors in human non-liver cells[36]. CD81 molecule on host cell surface acts as a viral receptor, that binds with viral particle and facilitates its entry in the liver cell[37,38]. CD81 is expressed on the surface of almost all nucleated cellsas a complex with a variety of other cell-surface receptors like CD19 and CD21 on B cells, andsends a costimulatory signal to the cells[39]. The viral envelop protein E2, binds to the major extracellularloop of CD8[[](http://www.jleukbio.org/cgi/content/full/76/4/743#B20#B20)40]. HCV shows multi-site binding and can also bind to several other molecules like the receptor for low-density lipoprotein, the dendritic cell (DC)-specificintercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN),and its liver counterpart[[](http://www.jleukbio.org/cgi/content/full/76/4/743#B23#B23)[4](http://www.jleukbio.org/cgi/content/full/76/4/743#B24#B24)1,42].E2 is the most variable viral protein and therefore,its interactions with CD81 have been reported to be strain-specific[[43](http://www.jleukbio.org/cgi/content/full/76/4/743#B25#B25)]. It has two hyper variable regions, HVR-1 and HVR-2 which undergo frequent mutations, possibly from virus-neutralizingantibodies and HCV-specific cytolytic T lymphocytes (CTL). HCV has a highmutation rate also due to the lack of proofreading abilityof its RNA-dependent RNA polymerase. Therefore, HCV exists inseveral distinct but closely related virus species within aninfected individual. These species are called HCV quasispecies.

**HOST IMMUNITY**

***Innate immunity***

Innate immunity presents a first line defense for control of HCV infections as it happens for several other viral infections. During HCV infection, cells produce Type 1 IFN that prepare and induce the cells to resist infection, check viral replication, promote adaptive immunity and activate Natural Killer (NK) cells, DC and Kupffer cells *etc*. Once inside the cell, the innate immunity *vs* HCV is triggered through host recognition of viral macromolecular motifs, known as pathogen-associated molecular patterns (PAMPs) as non-self by cellular pathogen recognition receptors. These receptors includes Toll-like receptors (TLRs) and Retinoic acid-inducible gene-I (RIG-I) like receptors (RLRs)[44]. RIG-I binds PAMP on HCV-RNA and activates interferon regulatory factor-3 (IRF-3) for expression of IFN-α/β and anti-viral/ interferon stimulated genes (ISGs)[45]. The secreted IFN and cytokines then activate Natural killer (NK), DC and Kupffer cell *etc*. These cells also play a significant role in mounting T/B cell based immunity[46]. PAMP region lies on 3` untranslated region (UTR) of HCV and induces RIG-1 signaling[47] that results in RIG-1 interaction with IFN-β promoter stimulator (IPS-1) causes activation of IRF-3 and nuclear factor κB (NFκB).

HCV can effectively evade innate immunity resulting in persistent viral infection. This is so because HCV has evolved to counteract the RIG-1 pathway[48] and thus evade the immune challenge. This phenomenon is the reason of chronicity in majority of HCV infected patients. For this, the non-structural proteins of HCV *i.e.,* NS3 and NS4A form a complex which activates NS protease domain to target cleavage of IPS-1. After cleavage, IPS-1 can no longer signal downstream to activate IRF-3 and NFκB and the infected cells no longer produce IFN-β or express ISGs[49].

NK cells, a major arm of innate immunity, play an important role in eradication of HCV. Liver is enriched in NK cells that usually become activatedin an early phase of a HCV infection. The activated NK cells recruit virus-specific T cells and induce antiviral immunity in liver. They also eliminate virus-infectedhepatocytes directly by cytolytic mechanisms and indirectlyby secreting cytokines including IFN-γ and TNF-α. These cytokines induce an antiviral state in hostcells. Surprisingly, HCV has evolved multiple strategies to counterhost’s NK cell response. It isinteresting to mention that whereas activated NK cells contribute toward liverinjury inactive or compromised NK cells permit the virus to continue invasion[50].

 ***Adaptive immunity***

After entry and replication of virus inside liver cell, the viral molecules are transported to the endoplasmic reticulum and get associated with major histocompatibility complex (MHC) molecules, which are finally transported to cell surface. These molecules on cell surface are recognized by T cells for their immune action. Majority of CTL are CD8+ and recognize antigen presented on MHC class I molecules. About 10% of CTL are CD4+ which recognizes antigen presented on MHC II molecules. These CTLs eliminate cells infected with virus. However, HCV have is reported to have evolved mechanisms to avoid recognition by CTL. They either reduce the expression of MHC molecules or prevent the viral peptide from presentation at the cell surface. Thus, CTLs play a major part in viral eradication[51] and immunopathogenesis of HCV infection[52].

In another pathway of mechanism, the destruction of HCV-infected hepatocytes release HCV fragments that are taken up by myeloid DCs. These DCs migrate to the draining lymph nodes and express HCV antigens on HLA class II molecules. Subsequently, they increase expression of costimulatory molecules (CD80, CD86) which interact with and activate antigen-specific helper T (Th) cells[53]. These activated Th cells promote the maturation of DCs and increased expression of CD40 ligand and TNF-α. The mature dendritic cells induce T-cell activation by over expression of their surface molecules. They also enhance antigen presentation capacity *via* HLA-I and production of cytokines that stimulate T-cell activation. IL-12 has been shown to play an important role in stimulating IFN-γ production from activated T cells[54-55], and thus, induces the development of type 1 (Th1) immune response characteristic of CTL activation.The effector CTLs release perforin, granzyme, and TNF-α, or express Fas ligand, and initiate a direct attack on HCV-infected hepatocytes[56,57].

The hepatocytes infected with HCV and DCs produce Type I IFNs which suppress viral replication by inducing enzymes such as 2′–5′ oligoadenylate synthetase (OAS) and RNA-dependent protein kinase (PKR) in hepatocytes[58]. The plasmacytoid DC recognizes HCV related markers through TLR-7, which interacts with single-stranded RNA[59]. The TLR-signaling up regulates PDC-Triggering Receptor Expressed on Myeloid Cells (PDC-TREM) that induce further production of IFN-α[60]. Activated OAS destroys viral RNAs, whereas PKR inhibits forming polysome of viral mRNA[58]. When HCV-specific CTL responses are not strong enough to eradicate the virus, it leads to persistent infection[61].

Successful clearance of HCV virus during acute HCV infection depends on the rise, vigour and sustenance of Th1 immune response[62,63]. Patients developing strong Th1 response showed efficient viral clearance and a self-limited course of disease. In contrast, those who lacked in IL-12 and IFN-γ production invariably developed chronic persistence of virus. Majorityof patients fail to control the infection and developa chronic infection with a variable degree of hepatitis andviremia[64,65].Experimental studies have also demonstrated that HCV components induce antigen processing mechanism and IFN-stimulated genes in the infectedlivers[66-68]. Impaired function of DCs, as antigen-presenting cells in inducing immunity, may be responsible for the impaired immune responses. Different studies have reported that the viral proteins including HCV core, E1, and NS3 inhibit DC maturation[69,70]. HCV infects DCs through the binding of HCV E2 protein and thereby suppress DC function in promoting antiviral effect[41,71].

The CTL activated by viral proteins, kill not only virus-infected cells but also contribute to virus control by noncytolytic mechanism throughsecreting cytokines, *e.g.* IFN-γ, IFN-α/β and tumornecrosis factor α (TNF-α). All these cytokines induce an antiviral state inhost cells. This also renders uninfected cells resistant to infectionand promote for stoppingviral replication. The progressionof the majority of the infected persons to chronic infectionsuggests the inability of the antiviral immunity to containthis infection. There may be several reasons for this failure,including emergence of escape variants as a result of a highrate of virus mutations, a decreased production of antiviralcytokines or "stunning" of HCV-specific CTL, a compromised cytolyticpotential of the CTL and antagonistic peptides[72].

This is important to note here that the HCV genome in single host is a dynamic population of different but closely related genomes, designated quasispecies. Generation of quasispecies is usually ascribed to high variation in hyper variable region-1 (HVR-1) during viral replication[73]. In acute resolving hepatitis, HVR-1 shows very little variation, as compared to that in chronic hepatitis[74]. HVR-1 induces anti-HCV neutralizing antibodies[75,76] and HVR-1 specific CD4+ and CD8+ T cells[77,78]. Using the responding host cellular immune response differentially, HVR-1 favours viral escape[79,80]. HVR-1 variations result from the action of a continuous immune-driven positive selection[81,82].Thus, HVR-1 complexity helps in virus adaptive strategy to escape the immune onset. HCV clearance is associated with a vigorous HCV specific CD4+ and CD8+ T cell response in the acute phase of infection. In contrast, viral persistence is associated with a weak and dysfunctional virus specific T cell response[79-83]. T cell failure and HCV immune evasion have been explained in several reports[84-86].

***Role of T regulatory cells in* a*daptive immunity***

Recent studies have suggested a possible role of different regulatory T cell populations in HCV persistence. These studies show higher frequency of CD4+CD25+ regulatory T cells in the blood and CD4+FoxP3+ T cells in the liver of chronically HCV infected patients[87-89]. CD4+CD25+ regulatory T cells suppress HCV specific CD8+ T cell and CD4+ T cell proliferation as well as CD8+ T cell IFN-γ secretion[87,90-92]. After HCV antigen stimulation Treg cells secrete IL-10 and Transforming Growth Factor- β (TGF-β) that suppress virus specific T cell responses[91-93]. CD4+CD25+ Treg cells obtained from chronically HCV infected patients demonstrated more suppressive activity against HCV specific CD8+ T cells compared to Treg cells isolated from acute HCV infected patients. However the suppressive effect observed in patients who successfully cleared the virus was still significant[90]. Another study showed that the frequency of CD4+CD25+FoxP3+ Treg cells and their suppressive capacity against virus specific T cell responses were as high in HCV recovered chimpanzees as in persistently HCV infected chimpanzees[94]. This observation still needs in depth studies to explore the actual suppressive effect of Treg cells during HCV infection. Induction of Treg cells by HCV antigens was demonstrated first time by a response of CD4+ T cell to HCV core protein. HCV specific IL-10 secreting T cells were detected in the blood of chronic HCV infected persons[95].The regulatory CD8+ T cells may play an important role in chronic HCV infection. HCV specific CD8+CD25+FoxP3+ T cells from blood of chronically infected patients suppress HCV specific T cell responses *via* TGF-β secretion. The blockade of TGF-β markedly enhanced the HCV specific IFN-γ secretion by CD4+ and CD8+ T cells[96].

Few other studies have shown that chronic HCV infection which brings an exhaustion or impairment of HCV-specific CD8+ T cells. During chronic HCV infection, CD8+ T cells show their failure to proliferate or secrete antiviral cytokines including IFN-γ. This phenomenon is promoted by lack of CD4+ T cells and expression of immunomodulatory cytokines like IL-10[97]. The major cause of HCV specific CD8+ T cells impairment is ascribed to expression of inhibitory receptor like Programmed Death-1, Lymphocyte-Activation Gene-3 (a protein related to CD4), CTLA-4 (a member of CD28 receptor family), T-cell immunoglobulin mucin-3 and 2B4 *etc*. on HCV-specific CD8+ T cells in blood and liver[98]. Expression of these inhibitory receptors is associated with low levels of CD127 expression and impaired proliferation and differentiation of T cells. Thus, different mechanism contributes to the dysfunction of HCV-specific CD8+ T cells in chronic HCV infection.

In addition to cytotoxic T lymphocytes, humoral immune response against viral and cellular components during HCV infection also shows its presence. Patients positive for HCV RNA and/or anti-HCVantibodies shows the presence of type I anti-liver kidney microsomeantibodies, which also recognize cytochrome P450 (CYP) 2D6. Thepatient’s liver is infiltrated with auto reactive mononuclearcells, which recognize CYP 2D6. It is interesting that the viralcore protein residues 178–187 bear sequence homology withhuman cytochrome P450 (CYP 2A6 and CYP2A7) residues 8–17[96].Although HCV is a hepatotropic virus and infects hepatocytes,viral genome and its replicative intermediates are frequentlypresent in the peripheral blood mononuclear cells andlymphoid tissues of chronically infected persons. The viral glycoprotein E2 has been implicatedin the oligoclonal expansion of several lymphoma cells[99].Themost common rheumatic and cutaneomucous symptoms in HCV-infectedpatients include fatigue, arthralgia, paraestheisa, myalgia,pruritis, and the sicca syndrome[100].

**ROLE OF VIRAL PROTEINS and GENOTYPES**

The role of structural and non-structural components of HCV virion has been explained with variation in their interactions with metabolites and affecting pathogenic pathways leading to liver damage. HCV-core protein has a prominent role in all these interactions as compared to envelope and non-structural proteins. Moreover, when the mechanism of this interaction was studied in relation to various HCV genotypes, it was observed that different genotypes behave differently to regulate all these pathogenic pathways.

The role of NS5A and E2 region was found be important. NS5A has a role in viral replication, inactivating PKR[101-104], blocking apoptic pathway, binding of growth factor receptor-bound protein 2[105,106] and induction of anti-inflammatory interleukin secretion[107,108]. Similarly, E2 protein inhibits PKR[109,110]. The region of NS5A that interacts with PKR, shows clustering of amino acid changes during IFN treatment and plays important role in evasion mechanism[111].Further, this association varies in genotypes and so, alters their sensitivity to IFN treatment. NS5A remains under strong immune selection, has T- and B-cell epitopes and possibly, in combination to individuals’ HLA, selects immune cells in a way so as to produce sensitivity/resistance to IFN therapy[112]. The functional activity of NS5A towards immune selection is clearly governed by the HCV-genotypes and varies accordingly. Response of genotype 2 and 3 to IFN treatment may be due to individuals recognizing the NS5A protein immunologically[13].

Binding of HCV E2 protein to DC induces their maturation. Several CV viral proteins, including core, NS3, NS5A and NS5B proteins, have been shown to inhibit DC functions[69]. Consequently, the functions of both CD4+ Th cells and CD8+ CTL are impaired in chronic HCV patients. This has been suggested to be one of the mechanisms that HCV virus utilizes to weaken host immune responses and spread the infection. Indeed many clinical studies have shown that in chronic HCV patients, not only the functions of DC are impaired[[113,114]](http://www.jleukbio.org/cgi/content/full/76/4/743#B26#B26), the functions of both CD4+ and CD8+ T cells are also impaired[[1](http://www.jleukbio.org/cgi/content/full/76/4/743#B26#B26)15]. Similar inductive effect of E2 protein was also reported on other cell types, including T cells, B cells[[1](http://www.jleukbio.org/cgi/content/full/76/4/743#B26#B26)16], hepatocytes[[11](http://www.jleukbio.org/cgi/content/full/76/4/743#B26#B26)7] and hepatic stellate cells[[118]](http://www.jleukbio.org/cgi/content/full/76/4/743#B26#B26).

The role of HCV genotypes in the progression of liver disease is one of the most controversial areas of HCV research. In patients with chronic HCV, infection with genotype-1b is reportedly associated with a more severe liver disease and a more aggressive course than the infection with other HCV genotypes*.* Similarly, it was found that HCV genotype-1b was significantly more prevalent among patients with liver cirrhosis and those with decompensated liver disease requiring liver transplantation than among those with chronic active hepatitis C[[119-121]](http://www.jleukbio.org/cgi/content/full/76/4/743#B26#B26). Although this is indirect evidence, it suggests an association between HCV genotype-1b and the development of these complications. HCV genotype-1b is a marker for more severe HCV associated liver disease, because it reflects a longer time of infection than a mere aggressive form of hepatitis C.

**METABOLIC CONDITIONS AFFECTING HCV PATHOGENESIS**

In addition to immune mediated HCV pathogenesis, there are several other clinical and metabolic conclusions having strong association with HCV pathogens. This include HCV induced insulin resistance, oxidative stress and hepatic steatosis. Following is the brief description of these conditions affecting HCV pathogens :

***HCV induced insulin resistance***

HCV infection influences overall metabolism leading to increased steatosis, fibrosis, inflammation, apoptosis and insulin resistance[122-123] during course of disease. The resulting insulin resistance shows a modulating impact on liver pathogenesis by HCV infection[124]. insulin resistance (IR) increases the *de novo* lipogenesis *i.e.* fatty acid (FA) synthesis *via* over expression and maturation of SREBP-1c. This in turn, increases the activities of lipogenic enzymes including Acetyl CoA carboxylase and FA synthase. At the same time, intermediates of triglyceride biosynthesis also activate inhibitors of insulin signaling. For example, activation of protein kinase C -E by phosphorylating insulin receptor substrate and thus inhibiting phosphatidyl inositol 3, 4, 5 triphosphate[125], inhibiting Akt translocation by ceramides *etc*[126]. HCV-core protein, either directly or via an increased secretion of TNF-α, causes IR[127,128]. The HCV core can activate inhibitors of insulin signaling including mammalian target of rapamycin[129] and suppressor of cytokine signaling (SOCS)-3 and C-Jun N-terminal kinase (JNK)[130,131]. The activation of JNK by HCV core may follow a direct or indirect proinflammatory cytokine mediated mechanism.

***HCV associated oxidative stress***

Oxidative stress is reported to be an important part of HCV-induced liver damage. Previous studies investigated the role of different molecular components of HCV structure in modulating oxidative stress during HCV infection. HCV-core protein present within the outer membrane of mitochondria induces oxidation of glutathione and promotes Ca2+ uptake into mitochondria. Clement *et al*[96] explained the molecular mechanism and demonstrated that following glutathione oxidation, there is increased reactive oxygen species (ROS) production by mitochondrial electron transport complex I and III. The HCV non-structural protein NS5A promotes ROS production in the membrane of endoplasmic reticulum (ER) by activating the release of Ca2+ from ER, thereby inducing oxidative stress[97]. NS3 protein induces ROS production by activation of NADPH oxidase[97].Increased ROS production and consequent oxidative stress is evident by presence of markers of increased oxidative stress in the blood. Levels of 8-hydroxy deoxyguanosine and 4-Hydroxy-2-nonenol are increased in HCV infection[132,133]. Similarly, few studies have shown reduced levels of glutathione during HCV infection. Another study shows that the serum level of thioredoxin, marker of oxidative stress, was significantly reduced in HCV infection[134-136].

Presence of oxidative stress has been noted in different types of hepatitis including hepatitis B. However, there is a remarkable increase in Oxidative Stress (OS) in HCV infection[132]. Several studies have shown that structural components of HCV induce an effective OS[132]. HCV-core and non-structural components NS3 and NS5A proteins directly induce OS[137-139]. Core protein is involved in OS generation *via* oxidation of mitochondria glutathione and uptake of Ca2+ into mitochondria[139,140] thus, changing the permeability of its membrane[141]. Electron transport complex I increases production of ROS and redistributes cytochrome from mitochondria to cytosolic fraction[93]. NS5A is associated with membrane of ER[142] and activates even signal transducers transcription and nuclear factor κB (NFκB)[107].All these activations lead to inflammation, immune response and apoptosis[143]. Similarly, NS3 triggers ROS by activating NADPH oxidase 2 in mononuclear and polymorphonuclear phagocytes[144] that increase role of apoptosis of hepatocytes[144]. All these reports finally concluded that the structural and non-structural components of HCV induce significant increase in OS that help in liver damage during HCV infection.

***HCV induced steatosis***

HCV infections isreported to have strong association with hepatic steatosis. There are several other factors also responsible for causing steatosis. These include alcohol consumption, obesity, diabetes, *etc*[145,147]. Studies on steatosis in relation to hepatotropic virus demonstrated that HCV infection directly causes steatosis in some patients[148]. Studies in experimental animals have shown that HCV-core protein promotes steatosis in liver[149,150]. Furthermore, when steatosis was studied in relation to HCV-genotypes, it was noticed that although steatosis is induced by all HCV-genotypes, it appears more prominent and frequent with HCV-genotype 3 infection[151-153]. In those patients carrying genotype-3 infection, there is a good correlation between level of steatosis and HCV replication[153,154] and presence of HCV-core in liver. Also steatosis disappears in patient with genotype-3 when treated successfully by anti-viral therapy as compared to those with non-genotype-3 who remain steatotic[155,156]. Steatosis reappears with relapse of infection[155].This clearly demonstrates that some HCV-genotypes have more steatogenic potential. Subsequent studies[157] indicated that genotype-3 interferes with very low-density lipoprotein (VLDL) secretion. Core protein, which promotes lipid accumulation in hepatocytes[158,159],proves more efficient from genotype-3 as compared core from genotype-1.

All these reports concluded that HCV causes steatosis in three different ways: (1) Impaired secretion of lipids from hepatocyte; (2) Increased *de novo* synthesis of free fatty acid (FFA); and (3) Impaired FA degradation. The first aspect of HCV-induced steatosis was proposed as due to the impaired secretion of VLDL. To substantiate it, reports from different studies demonstrated decreased level of Apolipoprotein B and cholesterol in chronic HCV infected patients[159,160]. Their low levels pointed towards HCV disturbing the assembly and secretion of VLDL from the liver[161]. Another important aspect in this relation was the evidence of increased *de novo* synthesis of FFA under the effect of HCV infection. In this context, it is suggested that HCV upregulated the Sterol Regulatory Element Binding Protein-1c (SREBP-1c) signaling pathway[158] with NS2 and NS4B proteins inducing SREBP at transcriptional level[162,163].It was also induced by expression of HCV core protein. Few studies in chimpanzees infected with HCV also demonstrated that HCV increase activity of lipogenic enzymes like ATP citrate lyase[164]. HCV-core, in particular, activates and helps in cellular lipid synthesis[164], possibly *via* its binding with retinoid receptor.

HCV-induced steatosis is also due to an impaired FA degradation by HCV. Expression of HCV-core protein is reported to reduce the expression of peroxisome proliferation activated receptor-α (PPARα), a nuclear receptor involved in FA degradation and down regulation of mitochondria β-oxidation[165]. Genotype-3 shows significant down-regulation of PPARα as compared to genotype-1[166,167]. It is again HCV-core protein that down regulates PPARα and so, is more effective when from genotype-3 as compared to genotype-1. Core protein from genotype-3 also down-regulated the PPARγ and upregulated SOCS-7 in Human Hepatoma cells (Huh-7) [167]. All these data clearly support that HCV-core protein may modulate the expression of various genes responsible for FA degradation via down regulation of PPARs.

**CONCLUSION**

the HCV infection that was previously known as blood borne non-A, non-B infection was found to be a serious public health problem of the world. The diagnosis of HCV is based on the detection of anti-HCV antibodies and/or viral nucleic acid in serum. The studies in last several years have developed assays for not only accurate serodiagnosis of infection, but also identification of HCV serotypes. The pathogenesis of HCV infection is quite complex and regulated by host immunity as well as several metabolic activities influencing liver function. Whereas both innate as well as adaptive immunity are involved in pathogenic action of HCV, the cytotoxic lymphocytes have a very crucial in deciding the eradication or persistence of viral particles. Moreover, the persistence of HCV infection is also affected by the viral proteins, HCV isotypes and liver metabolism. The problem of HCV pathogenesis still needs investigations to further understand it in more depth.

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**Figure 1 Proteins encoded by the** hepatitis C virus **genome.** Genome organization of hepatitis C virus showing the structure of the viral genome, including the long open reading frame encoding structural and nonstructural proteins, and 5` and 3` non-coding regions (NCRs). [Source: Monica A *et al*. *Expert Rev Mol Med* 2003; 5]



**Figure 2 Regulation of hepatitis C virus pathogenesis by host immunity and metabolic factors.** HCV: hepatitis C virus.