REVIEW ARTICLE

Hepatitis C and hepatocellular carcinoma: A review of natural history
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Abstract
The main causative factor for the chronic liver disease is infection with hepatitis C virus (HCV). Around the world, it has been estimated that almost 180 million people are carriers of HCV. Infection with HCV leads to hepatocellular carcinoma (HCC) that is a frequent cause of mortality in HCV-infected patient. Among the most common cancers, it is ranked fifth worldwide. The annually death rate of HCC patients being caused by HCV is approximately 1 million. Epidemiological studies have demonstrated that prolong infection with HCV is the main threat for the development of HCC. Keeping the knowledge about the causes of cirrhosis and development of HCC in HCV patients is consequently very important for improving treatment choices and health-care delivery. Effective precautionary measures that can prevent the progression of HCC have now been well illustrated. The perfect natural explanation of HCC pathogenesis is so diverse that treatment strategies are highly difficult. Therefore, in the case of nonmalignant hepatic disease follow-up of the patients and treatment options must take into account to prevent the progression to carcinoma. In this review, we have strived to describe natural disease course of HCV infection and the ways through which it progresses to malignant hepatic disease.

Introduction
Hepatitis is an inflammation of liver mainly caused by hepatitis C virus (HCV), found mostly in Asia, Africa, and other developing countries. HCV is the hepatotropic virus that leads to high rates of morbidity and mortality worldwide. It has serious sequelae, for example, it can lead to acute hepatitis, chronic hepatitis, or a chronic carrier state and ultimately to hepatocellular carcinoma (HCC). The majority of HCC cases in Pakistan are associated with HCV.

Worldwide about 175 million people were infected with HCV, and it was responsible for 500,000 to 1,000,000 deaths every year. HCV has been detected in high rates in South East Asian countries such as Thailand, India, and Malaysia. In Pakistan, the prevalence rate of HCV in women and child was 6.7% and 1.3%, respectively. In another study, HCV has been reported as 5% in Sindh, 6.7% in Punjab, 1.1% in Khyber Pakhtunkhwa, and 1.5% in Baluchistan. High rates of hepatitis have been detected in 40-60 years of age, and it is even higher at the age of 60 years, and males had higher rates of antibodies against HCV compared to females.

Worldwide, HCC accounts for 711,000 cases that comprise 70-85% of patients having tumor primarily from the liver. It has been reported as 5th and 8th most common malignancy in men and women, respectively. HCC occurs most likely by two main factors; they are hepatitis B virus (HBV) and HCV that cause 70% of HCC cases worldwide.

HCV has more potential to produce cancer of the liver (HCC) that made it different from HBV due to three important clinico-biological features. Research on transgenic mouse suggested an important role of HCV in liver carcinogenesis because the core proteins of HCV have an oncogenic potential. Core proteins of HCV have the capability to accelerate the synthesis of free radicals. Therefore, hepatocarcinogenesis is not just because of inflammation, this is the specific feature and activity of its core proteins.

Researchers are still wondering how an immune regulated liver inflammation generates the formation of HCC in the absence of following a viral infection. While the examination of cells shows that mechanisms that release the viruses, principally encapsulates the polypeptide and stores a large quantity of noxious surface antigen within the liver cells. This mechanism leads to hepatocellular injury of higher severity and longer duration that can be characterized by liver inflammation,
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Hyperplasia, deregulation in transcription, and genomic aneuploidy which may progress to neoplastic transformation.\(^{14}\)

This review focuses on HCV-related HCC, the role of chronic inflammation and pro-tumoral microenvironment in hepatocarcinogenesis and suggests new perspectives for therapeutic interventions.

**HCV**

HCV was first identified in the 1970s, when a large number of people were suffered by HAV and HBV infections following blood transfusion. Many years later, another type of hepatitis was identified that was also transmitted by blood and named as HCV. The genome of HCV was discovered in 1989 and consists of a single-strand RNA that possesses positive polarity.\(^{15}\) HCV infects only humans and chimpanzees and belongs to a member of the Hepacivirus genus and family Flaviviridae. The genome of HCV lacks a reverse-transcriptase enzyme and cannot assimilate into the patient genome. Therefore, insertional mutagenesis can be excluded from the list of underlying mechanisms of HCC due to prolong infection with HCV. HCV causes inflammation of liver cells but does not kill the infected cells. Immunity generated against HCV has capability either to resolves the infection in approximately 2-5 months or destroys the liver slowly, gradually causing the development of HCC.\(^{16}\) HCV-specific antibodies are detectable 8-20 weeks after infection, whereas HCV-specific T-cells are typically detectable 5-9 weeks after infection.\(^{17}\) Viral clearance or persistence depends on the complex interaction of virus with the patient immune system.\(^{18}\)

**Genotypes of HCV**

HCV is highly heterogeneous. Researchers have identified mainly six genotypes and no less than 70 subtypes distributed around the world.\(^{19}\) Although different strains do not differ in their virulence or pathogenicity the response to therapy varies with different genotypes.\(^{20}\) Considering the epidemiological data from Pakistan, genotype 3a is the most common type (55.10%), followed by genotype 1a, 3b, and mixed genotype (10.25%, 8.20%, and 5.08%, respectively).\(^{21}\) In Pakistan, there is a strong correlation of persistent infection with 3a genotype of HCV with the development of HCC.\(^{22}\)

**HCV genome**

HCV genome produces a large polyprotein that is proteolytically cleaved to produce 10 viral proteins [Table 1].

A single open reading frame (ORF) forms an HCV genome, and this ORF encodes a 3000 amino acid polyprotein. The ORF is bordered by 5′ and 3′ untranslated regions (UTRs) of 341 and around 230 nucleotides in length. The structural core proteins C, E1, and E2 are cleaved by the peptidases of endoplasmic reticulum of the host and after maturation in these organelle assembled as internal membrane-bound progeny virions. NS2 to NS5B are involved in polyprotein processing and viral replication.\(^{23}\)

Viral genome changes rapidly, therefore, effective immunity against HCV is difficult to obtain. In an infected person, about $10^{12}$ complete virions are produced each day with a rapid half-life of about 3 h. In this replication new quasi-species emerge. Immune system should be very effective against these newly generated mutagenic viruses. Unfortunately, 80% of HCV-infected patients progress to long-lasting infection; cirrhosis occurs in about 10-20% of persons. Liver cancer develops after a period of 20-30 years in 1-5% of cases.\(^{24}\)

**Risk Factors of HCV**

Table 2 shows the major risk factors for HCV infection.

**Pathogenesis of HCV infection**

HCV infects not only hepatocytes but also cells of other organs such as leukocytes and epithelial cells. Inefficient immune response ultimately leads to chronic inflammation, tissue

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**Table 1: HCV viral proteins**

<table>
<thead>
<tr>
<th>Structural proteins and nonstructural proteins</th>
<th>Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly basic protein</td>
<td>C</td>
</tr>
<tr>
<td>Envelope glycoproteins</td>
<td>E1 and E2</td>
</tr>
<tr>
<td>Small integral membrane protein</td>
<td>p7</td>
</tr>
<tr>
<td>NS proteins</td>
<td>NS2, NS3, NS4A, NS4B, NS5A, and NS5B</td>
</tr>
</tbody>
</table>

NS: Non-structural; HCV: Hepatitis C virus

**Table 2: Major risk factors for HCV**

| Low income, underprivileged, disadvantaged, needing social support\(^{26}\) |
| Persons who use injectable drugs, prevalence of HCV in this group is 67%\(^{24}\) |
| Heterosexual intercourse\(^{27}\) |
| Shared and re-used needles and syringes\(^{27}\) |
| Transmission when mother is HCV-infected. Risk is 17-25% among mothers with HIV infection\(^{27}\) |
| People with HIV infection\(^{27}\) |
| People who have had tattoos and ear piercing\(^{27}\) |
| Non-injection drug abuse (cocaine inhalation); alcohol\(^{27}\) |

HCV: Hepatitis C virus
remodeling through cell growth, apoptosis and/or necrosis and induction of oxidative stress. HCV proteins affect a wide range of cellular and humoral activities in host cells, including cell signaling, modulation of transcription and translation, rearrangements of cellular membranes, apoptosis, and chemokines production. It does not cause cytotoxicity directly in hepatic cells. Researchers have demonstrated that cryoglobulinemia, immune complex persistence, and autoimmune recognition are the underlying mechanisms for hepatic injury.

Cryoglobulinemia is characterized by the presence of a superfluous amount of proteins that are mostly immunoglobulins and changes into insoluble form at lower temperatures. Numerous diseases such as multiple myeloma and HCV infection has been accompanying with it. According to Brouet classification, it has been classified into three types. Plasma cell dyscrasias mostly related to Type I. Types II and III have been associated with HCV. While autoimmune diseases, for example, systemic lupus erythematosus, and rheumatoid arthritis are strongly related to Type III.

About 75% of HCV-infected patients have circulating immune complexes in HCV infection has been ascribed to an inability of the immune system to mount a protective response, or due to viral factors that allow immune escape via mutations in antigenic sites. Production of non-neutralizing antibodies explains both the uncontrolled viral spread and the formation of damaging immune complexes.

On the basis of molecular mimicry of HCV core sequence and cytochrome P450 2A6 and 2A7 an association has been detected between HCV and autoimmune hepatitis. HCV does not cause autoimmune hepatitis Type II but the fact is that liver-kidney microsome-1 (LKM-1) (microsomal antibodies) are found in some patients with persistent hepatitis. LKM-1 antibodies the serological marker of autoimmune hepatitis Type II that react with cytochrome P450 2D6, are also found in 0-7% in HCV patients. The targets of LKM-1 antibodies in HCV infection are specific epitopes of cytochrome P450 2D6 and many other microsomal proteins of 59 kd and 70 kd.

Immune System against HCV

The pathways through which HCV infection proceed depends on multifaceted virus/host collaboration.

Innate immunity

Macrophages, natural killer (NK) cells, and NKT cells are the components of innate immunity. They are found in large number in liver cells. Initially immune cells and epithelial cells control the replication and dissemination of viruses, and finally stimulate the development of adequate antigen-specific antibody, CD4+ T-helper (Th) cells and CD8+ cytotoxic T-lymphocyte (CTL) response. Pathogen-associated molecular pattern (PAMP) are the receptors expressed by infected cells, and immunity in contrast to HCV is generated when they are recognized by specific PAMP receptor expressed by specific toll-like receptors. This interaction mediates the early immune response of the host by activating latent cellular transcription factors. Nuclear factor κB (NF-κB), interferon (IFN) regulatory factor (IRF)-3, IRF-5, and IRF-7 are the factors which mediate these responses. NF-xB mediate the inflammatory response to HCV by inducing chemokines and proinflammatory cytokines in parallel with tumor necrosis factor-α (TNF-α).

IFN-β is released from infected hepatocytes that limit cell-to-cell virus spread. They upregulate the MHC-I and MHC-II molecules and initiate memory T-cell proliferation. IFNs have many other functions such as prevent T-cell from apoptosis, stimulate NK-cell activation and maturation of dendritic cells (DCs). Interleukin-2 (IL-2) and IFN-γ are the cytokines released by Th1 cells when they are exposed to specific antigens. NK-cells that was activated by IL-2 down-regulate the immune system by killing immature DCs. While IFN-γ production decreased in response to cytokines such as IL-12 and IL-15 and they may affect the development of Th1 responses favoring Th2 responses, thus permitting virus persistence and chronicity of the disease. That is why HCV infection precedes with the increase of Th2 cells and the chances of carcinogenesis increases.

Adaptive immunity

Specific CTLs expansion for viral clearance is mediated by adaptive immune components, i.e., CD4+ T and CD8+ T-cells. During the acute phase of infection CD8+ T-cells that has been exposed to HCV antigens is high (2-8% of peripheral CD8+ T-cells), whereas there is a fall in the frequency of CTLs when HCV continue to rise (0.01-1.2%). CD4+ T-cells produce cytokines for examples TNF-α, IFN-γ, IL-2 to effective CD8+ T-cell response that is necessary to protect the individual from HCV persistence. However, CD4+ T-cell responses are weak, short-lived, and they progressively disappear during the transition from acute to chronic infection. Cytotoxic cells function through four mechanisms: Cytolytic that causes the apoptosis of infected liver cells, noncytolytic that release IFN-γ, the release of cytotoxic granules such as perforin and granzyme B (cleave pro-caspases leading to apoptosis) and TNF-related apoptosis. Treg cells (CD4+CD25+) are the subpopulation of CD4+ T-cells, they produce IL-10 that acts as an anti-inflammatory cytokines and leads to impaired secretion of IL-2. Treg cells are found in high proportion in the peripheral blood and in the microenvironment of HCC patients.

Cytokines

Variations in cytokine manifestation levels play a noteworthy part in the progression of HCC. Proinflammatory cytokines, for example, IL-1β, IL-15, IL-18, TNF-α, TNF-αRs, TNF-αRI, TNF-αRII, and IL-6 levels have been found higher in patients with HCC as compared to healthy controls. While Th2 cells cytokine profile IL-4, IL-8, IL-10, and IL-5 play dominant role in HCC patients and they have been found to be associated with disease aggressiveness.
Progression of HCV Infection toward HCC

Chronic liver inflammation and cirrhosis are two most important causes of HCC. However, the large number of studies performed in the last decades led to the identification of main threats for HCC that helped to understand the pathogenesis of HCC. Viral infection or the exposure to hepatotoxic agents leads to significant changes in the cellular signaling pathways and their target genes that are responsible in the regulation of tumor formation. Some of these pathways include p53, mitogen-activated protein kinases, pRb, stress signaling, Ras, JAK/STAT epidermal growth factor receptor, Wnt/β-catenin, and transforming growth factor β1 (TGF-β). Wnt/β-catenin pathway has been found to be involved in the progression of HCC development caused by HCV. β-catenin pathway has been found to be upregulated in HCC patients. Thus, through the inactivated Wnt pathway we can consider it a potential therapeutic target for the prevention or the ablation of HCV-associated HCC. The tumor suppressor P53 gene, is the most important protein involved in tumor development which can be inactivated by single point mutation. Intracellular or extracellular stress signals can lead to significant changes in the expression level of P53 that resulted in up-regulation of P53. Retinoblastoma, pRb1 is a major cellular barrier to cancer development that controls cell cycle progression through a mechanism including, the repression of the E2F transcription factor family of proteins. There is a strong correlation between the loss of pRB and the inhibition of functional pS3 in cells which exhibit HCV core proteins. Therefore, it has been involved in different tumor types including HCC. Various studies have been reported in deregulated pathways of pRb in HCC. TGF-β1 is the most prominent profibrogenic cytokine that can be released from any cell type during inflammation, tissue regeneration, and fibrogenesis. It is an anti-inflammatory cytokine, but TGF-β1 is a well-known tumor promoter too. The mechanisms of tumor promotion include dysregulation of inhibitors of CDK, alteration in cytoskeletal architecture, an increase in proteases and extracellular matrix formation, decreased immune surveillance, and increased angiogenesis [Figure 2].

Current Diagnostic Modalities for HCV and HCC

One of the major reasons of liver transplantation in USA, Australia, and Europe is HCV. Anti-HCV antibodies and HCV-RNA are the diagnostic parameters for diagnosis of HCV infection. Procedures that are used to indicate localization of carcinoma inside liver include, magnetic resonance, computed tomography (CT), ultrasound and hepatic angiography with or without CT. However, biopsy remains the gold standard for confirmed diagnosis.

Assessing the degree of liver fibrosis and cirrhosis

Several liver biopsy-scoring systems have been developed, of which the METAVIR system is the most widely used, which is as follows [Table 3].

Non-invasive fibrosis tests

A variety of non-invasive fibrosis tests based on blood indices and imaging modalities are now available, which may be more suitable for underdeveloped countries. These include serum tests, for example, FIB4 scores and aminotransferase/platelet ratio index, which measure platelet count, alanine aminotransferase, and aspartate aminotransferase that are indirect markers of fibrosis. These tests should be available at all clinics treating patients with HCV.

Tumor markers

They are used for identification of HCC. Many tumor-related proteins, polypeptides, hormones, and isoenzymes have secreted by hepatoma cells into blood such as hepatoma-specific gamma-glutamyl transpeptidase and alpha-fetoprotein (AFP). Current specific markers include TGF-β1, free insulin-like growth factors (IGF)-β1, hepatoma-specific AFP subtractions. AFP-mRNA, IGF-II-mRNA, and TGF-β1-mRNA are the genetic markers used to observe metastasis reappearance of HCC after operation.

Treatment and Prognosis of HCV and HCC

Now, HCV is a curable disease, and with the passage of time advances in HCV therapy make cure rates higher. People who are cured from HCV cannot transmit the disease to others and they are more than 75% less likely to develop HCC. Until December 2013, six drugs were licensed for the treatment of HCV, standard IFN or pegylated IFN-α (PEG-IFN), the protease inhibitor boceprevir, simeprevir and telaprevir, the nucleotide analog polymerase inhibitor sofosbuvir and ribavirin (RBV). There are limitations of treatment such as high cost, the need for

Figure 2: Evolution from hepatitis C virus infection to hepatocellular carcinoma
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Table 3: METAVIR classification for staging of hepatic fibrosis

<table>
<thead>
<tr>
<th>Metavir stage</th>
<th>FO</th>
<th>FI</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition</td>
<td>No fibrosis</td>
<td>Portal fibrosis without septa</td>
<td>Portal fibrosis with septa</td>
<td>Numerous septa without cirrhosis</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

sophisticated laboratory tests and trained clinicians, as well as the limited efficacy and high toxicity of some of the medicines. Different types of HCV genotypes respond in a different way to treatments.\(^{27}\)

Current Therapies for Different Genotypes of HCV

The protease inhibitors boceprevir, simeprevir, and telaprevir, are used only for genotype 1. In HCV genotype 1 patients, high rates of sustained virological response (SVR; a negative HCV RNA test 3 or 6 months after the end of treatment) have been reported when being treated collectively with PEG-IFN, RBV, and a PI or nucleotide polymerase.\(^{43}\) Genotype 2 and 3 infected patients are treated by double therapy with PEG-IFN and RBV or sofosbuvir with RBV is used. Sofosbuvir, PEG-IFN, and RBV have shown to be similar response rates in genotype 4-infected individuals as in genotype 2 and 3 infected individuals. Genotypes-5 and 6 infected individuals are treated in the same way as genotype 2 and 3 and have shown high rates of SVR.\(^{41}\)

MicroRNA (miRNAs) as diagnostic tool in HCC

MiRNAs are small RNAs that do not encode proteins, always derived from an internal origin. MiRNA is found in many body fluids, and serum is one of them. Since they are resistant to RNAse activity, extreme pH and temperature, they are stable in serum. Some circulating miRNA levels increased in HCC patient serum and have a potential to be used as diagnostic marker of some diseases. High serum stability allows miRNAs to act as biomarkers for cancers, including HCC. For example, combination of miR-16, AFP, and DCP yielded the optimal sensitivity (92.4%) and specificity (78.5%) for HCC diagnosis. Therefore, it could used as a serum biomarker for HCC diagnosis. Further researches should be conducted for the application of miRNAs in treating HCC, and how to solve critical issues to interpret the miRNAs dysregulation. It is important to be able to identify dysregulation related to HCC as compared to other hepatic diseases.\(^{45}\)

miRNA-122 (miR-122) as a therapeutic agent in HCV infection

MiR-122 is vital for the constancy and proliferation of HCV RNA and is present abundantly in the liver. In highly conserved S’ UTR of the HCV genome lying a spaced labeled as S1 and S2 and miR-122 has capability for binding to it. This process results in the formation of oligomeric miR-122-HCV complex, that keeps HCV genome save from breakage and from natural immune system of the host.\(^{46}\) Among all the types and subtypes of the HCV genome, this binding site is preserved. That is why miR-122 could be used as a target in therapy against virus.\(^{47}\)

Miravirsen is a 15-antisense oligonucleotide that is modified in such a way that it is complementary to and has high specificity for the S’ region of mature miR-122. Therefore, miravirsen can isolate and inhibit miR-122. It has shown the reduction of RNA levels of HCV with no confirmation of viral resistance.\(^{48}\)

Management Opportunities of HCC

Anti-viral agents and chemoprevention methods play a major part to decrease the risk of HCC. IFN therapy is helpful in the prevention of HCC recurrence and improved survival, but mechanism through which it acts is unknown. Role of IFNs still remains uncertain because of uncertainty in inducing anti-tumourigenic or anti-inflammatory activities directly or indirectly. HCC can be treated by ablation therapy with ethanol/ radiofrequency waves, hepatectomy, liver transplantation, and transarterial chemoembolization (a minimally invasive procedure performed in interventional radiology to restrict a tumor’s blood supply).\(^{49}\) HCC progresses rapidly due to formation of large number of blood vessels, therefore, anti-angiogenic gene therapy may be used for the reduction of tumor growth, and is a new methodology to treat HCC.\(^{50}\)

Avenues for the Future Research and Conclusion

Chronic infection with HCV leads to cirrhosis and HCC. Cancer is a multistep process and expression of viral core proteins is essential for carcinogenesis. HCC that has been associated with HCV is correlated with liver fibrosis. Signal transduction pathways involved in the regulation of HCC associated with HCV can be a roadmap for the treatment or prevention of HCC. Lives can be saved through rapid identification, effective treatment and their referral to the specialized centers.

References


