Epidemiology, risk factors, and pathogenesis associated with a superbug: A comprehensive literature review on hepatitis C virus infection

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Abstract
Viral hepatitis is a major public health concern. It is associated with life threatening conditions including liver cirrhosis and hepatocellular carcinoma. Hepatitis C virus infects around 71 million people annually, resultantly 700,000 deaths worldwide. Extrahepatic associated chronic hepatitis C virus accounts for one fourth of total healthcare load. This review included a total of 150 studies that revealed almost 19 million people are infected with hepatitis C virus and 240,000 new cases are being reported each year. This trend is continually rising in developing countries like Pakistan where intravenous drug abuse, street barbers, unsafe blood transfusions, use of unsterilized surgical instruments and recycled syringes plays a major role in virus transmission. Almost 123–180 million people are found to be hepatitis C virus infected or carrier that accounts for 2%–3% of world’s population. The general symptoms of hepatitis C virus infection include fatigue, jaundice, dark urine, anorexia, fever malaise, nausea and constipation varying on severity and chronicity of infection. More than 90% of hepatitis C virus infected patients are treated with direct-acting antiviral agents that prevent progression of liver disease, decreasing the elevation of hepatocellular carcinoma. Standardizing the healthcare techniques, minimizing the street practices, and screening for viral hepatitis on mass levels for early diagnosis and prompt treatment may help in decreasing the burden on already fragmented healthcare system. However, more advanced studies on larger populations focusing on mode of transmission and treatment protocols are warranted to understand and minimize the overall infection and death stigma among masses.

Keywords
Direct-acting antiviral agents, epidemiology/public health, hepatitis C virus, infectious diseases, Pakistan, pathogenesis, risk factors

Date received: 20 October 2021; accepted: 20 May 2022

Introduction
In 1989, the prolonged scientific experiments resulted into the breakthrough of hepatitis C virus (HCV) origin and cause of chronic liver disease.1,2 Hepatitis A, B and C viruses were differentiated to each other by Feinstone et al.,3 evaluating that most of the transfusion related hepatitis is caused by neither Hepatitis A nor Hepatitis B virus. In recent decades following the discovery of HCV, efforts were made to advance the diagnosis of HCV infection, calculation of approximate viral titer in the blood, genotyping and elucidate the natural antiquity of chronic HCV infection.4–9 The precise worldwide

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burden of HCV infection can be assessed after the climax of HCV diagnostic practices.43

According to the etymology of word the “hepatitis,” it is a Latin word that refers to the swelling of hepatic tissues. Currently, viral hepatitis is considered as a major public health concern that is threatening mankind at major masses especially in the Asian developing countries like Pakistan.8 Viruses are playing major role in hepatic infections and leads to the liver associated morbidity and mortality. Life threatening conditions such as liver cirrhosis (LC), fibrosis, and occasionally hepatocellular carcinoma (HCC) may develop as the disease progresses from acute and chronic form to the more advanced stage.44

Chronic HCV affects around 71 million people annually that results into approximately 700,000 deaths per year globally. Prevalence of HCV varies among different areas and different groups of same population. Other than hepatic diseases such as cryoglobulinemia, glomerulonephritis, dysfunction of salivary glands, thyroiditis, pulmonary fibrosis, skin disorders, Behcet’s disease, fibromyalgia, polyarthritis, Guillain–Barre syndrome, thrombocytopenic purpura, ocular disorders, and other less frequent conditions are also associated with HCV infection.13–17

A massive load on healthcare system is implicated by the extra hepatic diseases that account for up to three quarter of the hepatitis patients.18 Healthy individuals or those treated to clear the viral infection get saved while those having chronic HCV infection resulted into enhanced non-liver associated mortality.19 In HCV infected patients, 20%-40% of the acute cases get treated while the remaining patients associated mortality.19 In HCV infected patients, 20%-40% of the acute cases get treated while the remaining patients become chronic carriers of this deadly virus.20 Chronic carriers have up to 30% risk of developing LC within 20–30 years. More than 90% of HCV patients are treated with direct-acting antiviral agents (DAAs) that prevents progression of disease in liver, meanwhile decreasing the elevation of HCC.21 Until the emergence of LC-related problems, the HCV infection remains asymptomatic.22 The occurrence of cirrhosis and other associated complications can be mitigated if the physician detects the initial HCV infection symptom and makes use of the available therapy.23

Genome

HCV genome comprises of approximately 9600 nucleotides.24–26 It contains only one open reading frame (ORF) which is made up of nearly 9000 nucleotides. Viral duplication and translation are facilitated by the 5' untranslated regions (UTR) which exist at the terminus of open reading frame.27 A polypeptide of approximate 3000 amino acids is produced and ultimately broken down by an amalgamation of viral and host proteases that forms structural and non-structural proteins. Three structural proteins have role in the synthesis of viral particles (glycoproteins, namely, C i.e. core and E1 and E2 i.e. envelope) and seven non-structural proteins play their role in the assembly, processing, and replication of virus (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B).27 HCV is classified as a member of Flaviviridae family belonging to the genus Hepacivirus.28 Viruses of Flaviviridae family have a homogeneous arrangement and their genome is positive (+) sense single stranded RNA. Upon entry into the host cell, viral genome works similar as mRNA and by being in alliance with acclimated membranes of cell, it provides a platform for replication of virus via complete mileage intermediate which is negative (–) stranded.29 According to the morphology, HCV is an enveloped virus29 and replicates in the hepatocytes.30 Replication of HCV relies on the liver-distinct microRNA-122 (miR-122).27,31 Nucleocapsid is about 30 nm in diameter, globular in shape, flanked by lipid envelope obtained from the endoplasmic reticulum (ER) of host membranes and is assembled by the genomic RNA which is linked with the core protein. HCV makes its way to hepatocytes with the help of two main glycoprotein E1 and E2 receptors which are present on the envelope.32 Under electron microscope, the size of the viral units produced by in vivo and in vitro methods is 40–80 nm in diameter.33,34

Prevalence and phylogenetic classification of HCV variants

In infected patients, HCV exhibits a very discerning feature. The viral population has an extensive genetic heterogeneity which occurs at various stages, either at any specific time or during the disease progression. This genetic heterogeneity has found in different patients across the globe leading to various strains/isolates, genotypes, and subtypes.35–37 HCV has a diverse prevalence worldwide and which varies with the areas of low, intermediary and upraised level.2 Almost 123–180 million people are found to be HCV infected or carrier that accounts for 2%-3% of the world’s population.38

In 1993, HCV was categorized into six main genotypes with notable subtypes by analyzing the phylogenesis of HCV fractional sequences obtained by a large number of samples derived from infected people worldwide.35–37 All the epidemiologically known HCV variants are present in the genotypes 1–6. The inspection of full-length open reading frame sequence further verified the sequence arrangement of the genotypes.39–42 Besides this, a seventh crucial genotype was also reported a few years back and this genotype is found to have a very low prevalence.32 Smith et al. (2014) asserted that there are seven main groups and 67 subtypes, and subsequently, many other subtypes were also validated owing to progressive sequence analysis performed on newly assessable ORF sequences.44 At global level and in certain population groups, the subtypes 1a, 1b, 2a, 2b, 3c, 3q, 4a, 4d, 5a, and 6a are well explicated.45–47 At amino acid and nucleic acid level, different major genotypes-associated genomes vary by almost 30%. The subtypes usually vary by almost 15% and up to 10% variation exists among contrasting isolates between the subtypes. Along with the possibility of
Developing an effective vaccine, HCV poses substantial recommendations for diagnostic and therapeutic purposes because of upraised level of genetic heterogeneity that exists among all the genomes. An ostensible diversity exists in the ecological distribution of globally present salient genotypes. Almost 30%–40% of all the infections belong to genotypes 1 and 3. The genotypes 2, 4, 5, and 6 are responsible for 9%, 8%, 1%, and 6% of the infections, respectively. Genotypes 1, 2, and 3 are leading causes of 90% of all the infections in Europe. In Australia, Japan, Europe, and United States, genotype 1 is the most prevalent. In Italy genotype 2 is pervasive. A significant dominance of genotype 3 occurs in different countries, like Pakistan has 22% dominance. Amid genotype 1, the subtype 1a occurs predominantly in Northern Europe while Southern Europe has 1b. Amid genotype 2, Northern Europe is having dominance of subtype 2b whereas the Southern Europe is having 2c as the most prevalent. For the first time, in Italy, a patient from Sardinia was diagnosed of having 2c subtype. Subtype 3a characterizes genotype 3 almost entirely. Because of emigration from Africa and Middle East, transmission of particular subtypes in the population of intravenous drug addicts, genotypes 4 and 5 have extended distribution. In Europe, there is a rising dominance of genotypes 3a and 4d owing to the spread among intravenous drug addicts. Consequently, genotype 3a is the causative agent for about half of total infections in various Northern European countries. In the emigrants of Southeast Asia, genotype 6 is present occasionally. Furthermore, genotype 4 is mainly present in Africa and Middle East but the subtype 4d was at first conceded in a Danish patient. In Thailand, less serious genotypes like 7a and 7b are present. There exists a very insignificant effect of HCV genotypes on the chronic HCV infections. Therefore, critical liver infections are associated with all genotypes and liver steatosis is primarily caused by genotype 3. It is acknowledged that genotypes are associated with response to interferon (INF)-based therapy. Genotypes 1 and 4 react inelegantly with the therapy contrary to the genotypes 2 and 3. Genotype 3 is the most arduous to be treated for novel-IFN free DAA based therapies.

High HCV prevalence causes and possible solutions: Pakistan as an example

In Pakistan, HCV infects up to 19 million people that accounts for almost 5%–10% of the population. Every year approximately 240,000 new cases are being reported. The disease emergence is striking and doesn’t seem to be fixed in near future. In countries that are situated near the border, the estimates of HCV infections are quite less. Being a bordering country, Iran is having HCV frequency of less than 0.5% despite of upraised assessment of injecting drug use (IDU). IDU is one of the major risk factors for HCV. Street barbers and blood transfusion can be devised as additional factors causing the increase of HCV infection in Pakistan. Various efforts are being made by provincial management and non-government organizations (NGOs) to develop standardized blood banks. Despite of all the preventive measures, the HCV infectivity is uplifting. A possible solution to the increasing HCV infections would lie in the understanding of the country’s map. Pakistan comprises of four major provinces including Punjab, Sindh, Khyber-Pakhtunkhwa, and Baluchistan. Maximum frequency of HCV cases exits in Punjab (6.7%) and Sindh (5%). Contrarily, Baluchistan and Khyber-Pakhtunkhwa are having less frequency of nearly 1% each. This conflicting prevalence between the four provinces is perceptible. Most populous and industrial provinces are having high level of HCV saturation, where the inhabitants are having better access to healthcare resources and ultimately the best possible results. Contradictorily, incidence of HCV increases in the districts that show elevated progress on Human Development Index, so the prevalence and healthcare approach seem to be related. It is prognosticated that 70% of novel HCV infections in Pakistan are related to regular medical measures which demonstrates that HCV frequency and healthcare approaches are associated with each other. In healthcare conditions, the most dominating factor in spreading HCV infection is the use of unhygienic syringes. Pakistan is having highest rate of injection usage worldwide with the disposal rate of 5–13 injections per capita annually.

Syringes in Pakistan are quite inexpensive, that is, nearly Rs. 2–7 (US$0.02–$0.07) per syringe. There exists no intimidation of syringe shortage in Pakistan. Awareness about acquiring HCV through injections do exist among the patients and standard principles of medical techniques are being followed by majority of the healthcare providers. The real problem lies in the absence of supervision, neglectful regulations, and inspections in private healthcare settings. Lack of guidance regarding the proper disposal and handling of syringes persists. The expertise to accurately execute the injections is sometimes deficient in the paramedics and medical personals. Moreover, open trash fields and municipal waste sites carries the used and disposed syringes from the few private or unregistered medical centers. All these situations are conducive for the spread of HCV. Finding a solution to this issue is quite challenging. HCV infection is not only preventable but also curable, as mentioned previously. There exists a sequential incidence of HCV in developed countries. The finest procedures being followed in these countries can also be practiced in Pakistan and other high HCV prevalent countries. Initially, in order to ensure the correct use and disposal of syringes, it is necessary to educate and train the medical personals, paramedics, and medical associates. Second, ensure the proper disposal of syringes and imposing a conscientious ban on repackaging. Third, current healthcare system can be incorporated with advanced HCV facilities and the healthcare personals should...
be trained for a short period of time about the progressive diagnosis, preliminary therapy, and outreach. Fourth, introduction of sustainable treatment to stop the transmission of HCV. Finally and the most importantly, boosting up the public consciousness about the spread of HCV infection by the provision of helpful information through interagency cooperation among healthcare organizations, government, and communities. This review explains the wide range spread of HCV in populous areas and highlights the risk factors that contribute to the chronic HCV infection in developing countries. Pakistan is one of the developing countries affected by HCV on massive scale, and it also poses threat to humans worldwide. World Health Organization (WHO) plans to eradicate HCV from world by the end of 2030. The frequency and worldwide prevalence of HCV can be mitigated by early identification and elimination of risk factors.

**Life cycle of HCV**

The percentage of HCV positive cells found in sick liver tissue varies from less than 5%–100%. This can be correlated to virions generation rate of 50 units per hepatocytes per day. HCV is also able to replicate inside the secondary mononuclear cells of blood. Duplication cycle of HCV occurs within the subsequent fashion discussed below and depicted in Figure 1.

**Attachment and cell entry**

HCV life cycle begins when the infectious particle attaches to host cell and explicit *in vitro* interaction between CD81 receptor (located superficially on the host cell) and the viral attachment protein (E2 glycoprotein on the outside of the particle). CD81 has been recognized a receptor for other viral particles as well. This interaction is really an essential step for a virus to start an infection. For penetration into the cell, HCV needs to attach to the low-density lipoprotein (LDL) receptors. E1 is implicated inside the union of membrane. E2 operates as a chaperon for E1, therefore, when E2 is unavailable then E1 makes misfolded clusters.

**Polyprotein translation and processing**

The translation of the genomic DNA is instantly started as it sets foot in the cytoplasm. RNA translation is arbitrated by internal ribosome entry sites (IRES) rather than by a Cap-dependent method. There are various aspects which influence the function of HCV IRES. First, the IRES-dependent translation of the X-Tail is accomplished which is present at the farthest 3′end of the HCV genome. Second, to activate the translation, several cell features bind to the IRES including polypyrimidine-tract-binding (PTB) protein, the La antigen, heterogeneous nuclear ribonucleoprotein L and other unknown proteins. Translation of the polypeptide is performed at endoplasmic reticulum and cut co- and post-translationally by the host cell signals and two viral proteinases. The foregoing hydrophobic sequences to the splitting sites are cleaved at the N-terminal region at the C/E1, E1/E2, E2/p7, p7/NS2 junctions. The non-structural protein (NS) NS2-3 protease carries out the dispensing between NS2 and NS3 by means of prompt intramolecular reaction. NS3 domain binds zinc which has a crucial role in catalysis. The HCV proteins form a higher-order stable compound linked to intracellular membranes, whereas active enzymatically by itself. The proteolytic activity of NS3 is essentially activated through NS4A.

**RNA replication**

The synthesis of minus (−) and plus (+) strand RNA is primarily catalyzed by NS5B RNA dependent RNA polymerase (RdRp). A 3′ end is produced by intramolecular back folding or hybridization of the sequences at the 3′ end which is exploited for the elongation. NS5B can produce RNA primer due to the elevated concentrations of the GTP or ATP. Full-length genome of HCV is replicated by NS5B in vitro. However, other viral or cellular factors are also mandatory in vivo. The NS3 helicase is a likely viral candidate that keeps RNA template stable and aid in duplication of the NS5A phosphoprotein implicated for the management of RNA replication. Furthermore, PTB collaborates with the sequences present at the 3′ non-translated region (NTR), Glyceraldehyde-3-phosphate dehydrogenase (G3P dehydrogenase), interacts with the poly (U)-sequence in the 3′ NTR and the p87 and p130 cellular proteins. HCV replication might also be obstructed by proteins from other viruses, for instance elevated load of Epstein bar virus (EBV). This happens probably due to the stimulation of transcription of the cellular genes.
Virion assemblage and liberation

The core proteins provoke the maturation of the virion particle and RNA genome assembly. This attachment not only execute a peculiar covering of the plus (+) stranded genome but also seems to restrict translation of IRES. The nucleocapsids of the virus get their envelopes from ER membranes by the virtue of E proteins which have a distinct feature of their hold in the ER compartment. Under these circumstances, the constitutive secretory pathways might be employed by the virus for its transit. Partly filtered virus particles have obscure N-linked glycans on their surface which indicates the viral transit via Golgi apparatus.

Pathological consequences of HCV infection

The HCV infection results in several pathological conditions in the patients depending upon their immune capability. Some of the liver associated diseases arising as a result of HCV pathogenesis are discussed in this section.

Steatosis

Patients suffering from chronic HCV often suffer from a histological feature called as liver steatosis. Also known as fatty liver disease, this condition is characterized by too much fat build up in the liver. Two major factors, that is, genetic and epigenetic, play a contributing role in the developing link between hepatic steatosis and HCV. HCV can alter the intrahepatic metabolism of lipid by affecting lipid peroxidation, lipid synthesis, insulin resistance, assembly and secretion of very low-density lipoprotein (VLDL) and oxidative stress. The host-mediated and viral factors serve as major contributing factors for the hepatic steatosis buildup. In order to complete its life cycle, replication of HCV depends on the lipid metabolism of host. This leads to the formation of hepatic steatosis via various processes such as defacement of lipid oxidation in mitochondria, down pressing the microsomal triglyceride transfer protein (MTTP) activity and enhancement of lipogenesis.

Steatosis frequency varies with the genotype, and it occurs in almost 40%–80% of the patients suffering from chronic Hepatitis C. In case of genotype 3 infection, steatosis is more common and occurs in approximately 73% of the patients. However, the prevalence of steatosis is almost 50% in case of other genotypes. There exists a noteworthy relationship between viral load of HCV RNA and degree of steatosis. Development of steatosis in chronic HCV is affected by various factors such as viral factor (e.g., HCV genotype 3), drug therapy factors (like corticosteroids methotrexate and amiodarone) and host factors (such as being overweight, diabetes mellitus, insulin resistance, alcohol consumption, and hyperlipidemia).

Fibrosis

Liver fibrosis is the development of imprudent fibrous connective tissue that comprises extra cellular matrix proteins such as collagen fibers emitted by the activate hepatic stellate cells. It is mediated by wound healing response in response to due to tissue damage by chronic HCV infection. Liver fibrosis is a considerable complication of HCV infection, and its continuation can cause life threatening conditions such as liver failure, LC and hepatocellular carcinoma. For the treatment of liver fibrosis, viral eradication can contribute to decrease the liver damage by ameliorating the inflammation process and retrogressing the fibrosis regardless of the treatment method. In clinical practice, liver biopsy is being replaced by non-invasive methods, but their effectiveness for monitoring the fibrosis posttreatment with sustained virological response (SVR) still needs to be determined.

Cirrhosis

LC is the development of a censorious period during chronic liver disease caused by HCV. Without the provision of antivirus therapy, nearly 67%–91% of the patients have to face death because of other liver disorders such as liver function failure and liver cancer. Cirrhosis is a condition in which the normal liver structure exhibits disruption resulting from fibrosis and a nodule is generated that obstruct the normal functioning of liver. Old age, chronic HCV infection and excessive alcoholism serves as the risk factors for cirrhosis. After onset of HCV infection, cirrhosis takes almost 30 years on an average to develop. This average also significantly varies from person to person. Almost 4% annual deaths worldwide are caused by cirrhosis. It is estimated that Pakistan has the second highest HCV prevalence. In developing world including Pakistan, HCV has been documented as a major cause behind cirrhosis. There exists a very high HCV to cirrhosis conversion ratio in Pakistan. At present, almost 10 million HCV patients in Pakistan are at a risk of developing cirrhosis. Cirrhosis can further lead to severe illness such as portal hypertension, ascites, variceal hemorrhage, and most commonly rectal varices. Severity of cirrhosis may cause esophagus varices. Patients suffering from cirrhosis and hypertension have 30% risk of developing hemorrhage and bleeding conditions.

Hepatocellular carcinoma

Hepatocellular Carcinoma (HCC) is a heterogeneous and most common malignant tumor group that varies in genetic and epigenetic alteration events and risk factors. HCC is considered as the most frequent cause of primary liver malignancy and a main cause for worldwide cancer-related death. HCC is the ninth leading cause of deaths in United States. In last 15 years, there is an increase in the mortality rate linked with HCC. Incidence and mortality associated with
HCC continue to increase despite of the advancements in prevention, screening, diagnostic, and treatment methods. Regardless of etiology, cirrhosis plays an important role as being a significant risk factor for HCC development. Male population is more vulnerable to cure HCC than female population.106

**Immunopathogenesis of HCV**

An effective immune response toward HCV infection is hindered by multitude of viral proteins including core,107 E2,108 and NS5A protein.109 Both the innate and adaptive immunity play role in immunopathogenesis of HCV. The transition of acute to chronic HCV is also dependent on the interplay of immune modulations. The infection of hepatocytes by HCV leads to induction of cellular and adaptive immune responses. This section considers immunopathogenesis of HCV infection and chronic HCV infection development.

**Innate immune response against HCV**

The activation of innate immunity in reaction to the HCV invasion plays a crucial role in controlling the virus spreading. It causes apoptosis of hepatocyte which controls the virus progression. In addition, it induces the adaptive immunity as well.110 In acute HCV infections, the human cytoplasm contains the viral RNA genome. Following the virion’s uncoating, intracellular RNA genome of the virion induces the production of Toll-like receptor 3 (TLR3), Retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) inside the infected liver cells. This leads to the production of Type 1 Interferon (IFN-I, α and β) and IFN-γ.111 Plasmacytoid dendritic cells (pDCs) can also detect circulating viral RNA. IFN-γ is produced by the active pDCs. Both IFN-I (α and β) and IFN-γ produced by infected hepatocytes and circulating pDCs suppresses HCV multiplication and activate the natural killer (NK) cells directly. By cytolytic death of infected hepatocytes and production of cytokine, NK cells play a critical role in the innate immune response toward acute HCV infection, suppressing HCV replication and inducing the adaptive immunity. Infected hepatocytes undergo perforin and granzyme B-mediated apoptosis stimulated by the activated NK cells which also causes harm to adjacent normal hepatocytes. IFN-γ and Tumor necrosis factor (TNF)-α, which promote dendritic cell maturation, are also generated by NK cells. This causes the release of IL-12, which activates adaptive immunity by inducing CD4 and CD8T cells to differentiate and mature.112 In vitro research has revealed that IFN generated by NK cells suppresses HCV replication directly.111,113

**Adaptive immune response against HCV infection**

The key mechanism of viremia regulation in the adaptive immune system is T cell response.114 HCV-infected hepatocytes are destroyed by specialized CD8+ T lymphocytes through human leukocyte antigen (HLA) class I antigen presentation cells and by cytokine production (TNF-α, IFN-γ). This occurs when IL-2 stimulates NK cell and CD8+ T cell activation, which is supported by helper CD4+ T cell. Viremia lasting for 6 months or more, is considered persistent HCV infection.115,116 HCV uses a variety of methods to evade the immune system, resulting in immune system evasion and infection persistence.117 T cell function is lost in chronic T cell stimulation, which is the first main mechanism of chronic infection. T cell activity is essentially hampered, and their cytotoxic potential is diminished. Chronic viral antigen generation causes persistent activation of T cells resulting in the T-cell dysfunction. HCV-specific CD4+ helper T cells produce less IL-2 during the persistent infection, hindering activation of CD8+ T cell.118

**Immune system and chronic HCV infection**

In case of chronic HCV infection, the activity of CD4+ T cells is essential. In acute infection of HCV, a robust CD4+ T cell response is linked to viral clearance. However, the decline of the CD4+ T cell activity specific to HCV is closely linked to the transition of acute infection of HCV into the chronic HCV infection.116 Moreover, inadequacy and the subsequent reduction in a strong CD4+ T cell activity following acute infection have been documented and linked to chronic infection.119 HCV escape mutations for individuals having multiple HLA epitopes (plus the HLA-DRB1*15 epitope) have been hypothesized as one route which decreases the CD4+ T cell activity.120 CD25+ T cells are the regulatory T cells (Tregs) that help to decline immune system activation. During chronic HCV infection, these cells limit the immune response by a variety of methods, including the suppression of CD8+ T cells and the reduction of cytokine production, for example, transforming growth factor-beta (TGF-β), immunomodulating cytokines and IL-10.121 Chronic HCV is caused by viral escape mutations mainly, which appear in the initial phases of the acute infection and persist for many years in the quasispecies, indicating them as an immune system evasion strategy and persistent development.122 Virus epitopes targeted by CD8+ T lymphocytes are mutated in 50%–70% of the individuals with persistent HCV infections.121

**Transmission and risk factors**

**Barbers**

Barbers are identified as the most likely source of HCV transmission. Inadequate barber hygiene practices can spread HCV to clients. Razor blades can sustain the virus for a few days.123 Unsterile blades and razors, contaminated with virus-containing blood can profoundly transmit it to another person. Barbers are declared as the prime risk in HCV dispersal by a number of researchers.124,125 It is manifested that barber confer considerable prevalence rates.126
About 17.9%–24.7% prevalence rate was documented by some studies. Another study mentioned 58.6% risk among barbers. Barbers’ awareness and familiarity with viral hepatitis was also accessed in some studies. Hepatitis is enlightened as the liver disease and unhygienic razors are the prime cause of their dissemination by only 13% research. An intervening study from Islamabad documented that ample degree of awareness exist among people regarding the dissemination of viral hepatitis. More than 90% of the participating individuals had knowledge about the spread of HCV infection via reused blades. Comparing the level of awareness between rural and urban regions, it was found that acquaintance and knowledge was about 92% in urban regions whereas it was 68% in rural regions.

**Recycled syringes**

In the developing countries, reuse of utilized medical syringes in dispensaries, health centers, and healthcare workers is more frequent among families and people with low socioeconomic position. The children of such families are usually involved in the marketing of recycling junk and hospital waste and are at an upraised threat of infections. According to a nationwide survey of 2007–2008, it has been estimated that 86% of the women acquired their very last injection from unopened packs. Among medical care items which can be recycled, therapeutic injections are the second highest.

**Intravenous drug users**

Human socialization in Pakistan and several other developing countries have made intravenous drug use as the prime risk factor to disseminate this viral disease. In regard to a study, IDUs are 46 times more vulnerable to be HCV-positive than healthcare personnel.

**Transfusion of contaminated blood products**

Blood borne pathogen’s dissemination is mediated by the two main risk factors which are blood donation and transfusion. Literature also proficiently considers it as a threat factor for HCV. In regard to a study, IDUs are 46 times more vulnerable to be HCV-positive than healthcare personnel.

**Sexual transmission**

Sexual means can also transmit viral hepatitis. Unprotected sex and sexual affairs with multiple partners are the major cause of spread. Majority of the studies performed in Pakistan have concluded it a well-recognized and pronounced mode of transmission. Homosexuality is also substantially associated with the elevation of the disease. Extramarital affairs can also be one of the risk factors for viral hepatitis.

**Ear and nose piercing**

Blood borne pathogens are disseminated via the activities that could lead to blood wounds or seepage. In developing countries, females are more tend toward ear and nose piercings. Therefore, they are at a greater risk for imparting the disease. Usually, unsterilized instruments are being used by the people who do ear and nose piercings. A study had reported 11.7% occurrence of hepatitis due to ear and nose piercings.

**Healthcare workers**

Viral hepatitis is detected in all the population groups, but it predominates in a few peculiar groups, which are known as the high-risk groups. Two studies have reported the incidence of 3.41% and 4.13% as the HCV incidence among the healthcare employees and it was found to be maximum.

**Surgical procedures**

Dental surgeries integrate techniques that are prone to needle stick wounds and incur a high probability of the blood infections. Dental surgeries also involve procedures, for instance, use of unsterilized tools that can aid in disease spread. In addition, other surgeries are also a risk factor for viral turnover. Some causes of disease dispersal include inadequate prerequisites for blood, blood-related products, and the inexperienced conduct of the clinicians during surgeries.

**Vaccinators**

As vaccinators are engaged in a lot of vaccination projects which comprise the employment of injections, they could be a possible risk factor for viral dispersal. Occasionally, in the course of vaccination, virus dispersal can happen from contaminated to uninfected individuals.

**Perinatal transmission**

Transmission of blood borne pathogens occur in the procedures like child delivery as the interior organs are exposed which makes a person more susceptible to various infections. Caesarian operation is an anticipated risk. It has been documented that a caesarian operation is a principal element in Afghan refugees accommodating the slum areas in Pakistan, which had infected female employees. Such scenario might also be presented in other developing countries.

**Symptoms of HCV**

Fatigue, nausea, vomiting, abdominal pain under lower right ribs, pale stools, decreased appetite, low-grade fever, dark urine, joints pain, yellowing of skin and the sclera (jaundice) and tickling sensation are the most recurringly perceived...
Symptoms of the HCV are categorized into three phases discussed below.

**The prodromal phase**

Some patients feel sickness, which includes fever, arthralgia, arthritis, rashes, and angio-neurotic edema before the proper disease development. These symptoms end before jaundice, which is the most common and peculiar symptom of HCV.

**Pre-icteric phase**

In this phase, the patient develops respiratory problems and gastrointestinal tract disorders which may include malaise, fatigue, myalgia, nausea, and vomiting, which may be escorted by weight loss, headache, coryza, fever, or pharyngitis and cough. The pre-icteric phase lasts from 2–3 days to 2–3 weeks.

**Icteric phase**

Patients develop gastric pain, right upper quadrant discomfort, or diarrhea in the icteric phase. Darkening of urine and light-colored stool are observed in victims. Worsening of starvation, nausea-color vomiting, scratching and irritated skin lesions related to intense itching are the most peculiar symptoms of hepatitis that develop during this phase. Table 1 classifies specific and non-specific symptoms of HCV in icteric phase.

### Table 1. Specific and non-specific symptoms in icteric phase.

| Non-specific symptoms | Specific symptoms |
|-----------------------|-------------------|
| Flu                   | Fatigue           |
| Fever                 | Jaundice          |
| Arthralgia, rash      | Dark urine        |
| Arthritis             | Lack of appetite  |
| Angio-neurotic        | Bruising or bleeding |
| Edema                 | Vomiting or nausea|
|                       | Liver failure     |

Diagnosis of HCV

Diagnosis alludes to determining the type of disease or other problems by looking at signs and symptoms. Two major categories of tests are used to diagnose HCV. These tests include serological assays and molecular assays. For detection and quantification of HCV genome, molecular assays are being used, and antibody titer against HCV is determined by serological assays.

**Serological assays**

Hepatitis C is diagnosed using the HCV Antibody test. The enzyme immunoassay (EIA) is used to detect antibodies against the HCV in the patient’s blood or serum, and its third generation provides 99% accuracy. The results of this test do not indicate whether the infection is acute, chronic, or resolved. After the detection of antibodies, further confirmation of the virus should be done with the help of an HCV RNA test. The most common examples of serological assays are:

1. **Screening Tests for anti-HCV.** Its common example is Enzyme Immunoassay (EIA)
2. **Supplemental Tests.** Example: Recombinant Immune Blot Assay (RIBA)

For the detection of anti-HCV, three generations of tests have been developed till now and each one is more advanced and sensitive than the previous one. Antigens from the HCV core, nonstructural (NS) 3, NS4, and NS5 genes are involved in Enzyme Immunoassay 3 and Recombinant Immune Blot Assay 3.

**Molecular assays**

The most reliable method of HCV detection is to use polymerase chain reaction (PCR) to detect HCV nucleic acid (RNA) in the patient’s plasma or serum. It is well established that qualitative assays are more sensitive than quantitative assays. With sensitivities of 10–50 IU/mL, PCR and transcription-mediated amplification (TMA) assays have rendered qualitative assays simpler and more precise. The most sensitive HCV PCR assay currently available has a sensitivity of fewer than 100 copies of HCV RNA per milliliter of plasma or serum. The two main methods for determining HCV RNA levels are discussed here.

**Qualitative HCV RNA.** The qualitative HCV RNA tests give an all or none answer, indicating whether or not the virus is present in the patient’s body. The amount of virus in the patient’s body is not indicated by this test.

**Quantitative HCV RNA.** The quantitative HCV RNA test determines how much HCV is present in the body. This test will also tell you whether your infection is acute or chronic.

**Rationale of screening and molecular tests**

The identification of antibodies against HCV in a patient’s blood is the most widely used test for HCV, but the findings may be ambiguous and require careful interpretation. If antibodies against the HCV are present, it indicates that the individual is a chronic HCV carrier (75%–85%), has been infected in the past but the infection has subsided (15%–25%), or has been recently (acutely) infected. After HCV infection, the body needs at least 6–8 weeks to form enough antibodies to be tested in a screening test. For example, after.
being exposed to HCV, a person who is immunocompromised (e.g. has HIV infection) can have negative test results for nearly 15 weeks–6 months. An antibody test cannot detect an infection that has been present for less than 6 months. Antibodies in a person’s blood indicate that he or she has been infected, but this does not necessarily imply that the person is still infected. Within 6 months of being exposed to HCV, up to 25% of people can remove it by the action of their defense system. The most widely used follow-up test is the qualitative HCV RNA test. The virus’s genetic material is RNA, which is detected by the qualitative examination. The titer of the virus is determined by a quantitative RNA test, also known as a quantitative viral load test. Some medical care providers demand a follow-up test before disclosing the results of an HCV antibody screening test to their patients, owing to the difficulty in interpreting the test. HCV infection is considered chronic if HCV RNA has been present for at least 6 months. Negative HCV antibody test results have a high degree of accuracy. IDUs and people who are involved in other high-risk activities should, however, be retested every year to account for the 6 month window phase.147,150

### Treatment

Patients with chronic hepatitis C are given antiviral therapy except for those patients who have co-morbidities. Treatment for HCV is increasingly improving and is successful. According to the Canadian Agency for Drugs and Technologies in Health (CADTH), treatment of HCV with interferon-free direct-acting antiviral agent–based therapy is successful against all stages of fibrosis.

#### Pretreatment assessment of patients

Questions regarding the patient’s life after antiviral therapy, as well as other factors such as the duration of infection, signs, and symptoms of disease, and the existence of cofactors that can intensify disease (e.g. alcohol, obesity, co-infections), are asked before treatment. To confirm the amount of HCV RNA and its genotype, pretreatment tests are done, and these tests involve liver biochemistry and function, abdominal ultrasound, fibrosis stage assessment, and tests to rule out co-infections.

#### Table 2. Genotype-specific drugs and respective treatment duration.

| Genotype | Drugs | Weeks |
|----------|-------|-------|
| 1a       | Ledipasvir/sofosbuvir | 8–12 weeks |
| 1b       | Elbasivir/grazoprevir (Zepatier) | 12–16 weeks ± ribavirin† 8–12 weeks |
| 2        | Sofosbuvir + daclatasvir (Sovaldi + Daklinza) | 12 weeks |
| 3        | Sofosbuvir/velpatasvir (Epclusa) | 12 weeks |
| 4        | Glecaprevir/pibrentasvir (Maviret) | 8 weeks |
| 5        | Sofosbuvir/velpatasvir (Epclusa) | 8 weeks |
| 6        | Glecaprevir/pibrentasvir (Maviret) | 12 weeks |

†Ribavirin is administered along with Zepatier based on the genotype, baseline resistance mechanism and treatment experience.154

#### Treatment routines

Patients who have never received HCV medication are treated for different periods of time in weeks, depending on the genotype of the HCV. Table 2 explains the drugs used for specific HCV genotype and the duration of the treatment.151–153

#### Post-treatment

Patients who do not reveal any more signs and symptoms of the virus do not require post-treatment, although those with alanine aminotransferase elevation or constant risk exposures (e.g. people who inject drugs) should have annual HCV RNA testing. Patients with cirrhosis and who have had a viral response should be screened for hepatocellular carcinoma regularly. Cirrhosis patients need hepatocellular carcinoma with biannual ultrasound before treatment. Rescue treatment should be provided to patients who have not responded to the viral treatment. Patients who do not get a viral response due to adherence problems or drug-drug interactions should be treated with caution. For 12 weeks, a single-tablet regimen of sofosbuvir, velpatasvir, and voxilaprevir is effective against all genotypes of HCV.151–153

#### Conclusion

HCV infection is a complex systemic disease with serious medical and economic consequences. It is important to assess the full range of HCV disease burden to fully comprehend its impact on patients and the general public. For instance, considering Pakistan as a developing country representative, it is experiencing a historic HCV epidemic, with one out of every 20 citizens previously being infected with the disease which is placing a significant burden on the country economics and healthcare settings. A rapid increase in HCV seroprevalence among the individuals who had previous surgical and medicinal treatments, suggests a major role of hospital-acquired infections in the spread of HCV. Some of the main causative risk factors include needle prick- ing, barber shaving, blood and its products, dental procedures, IDUs, unsafe delivery methods, dialysis, and vertical transmission from mother to baby. To minimize the risk of
HCV infection, it is suggested that proper precautionary measures should be implemented.

Although efforts are being made to increase the coverage of safe injections, blood examinations, advanced infection management, and assurance of prevention and safe practices in all sectors of healthcare organizations must be assured to accomplish the HCV elimination goal by 2030. However, there is still a long way to go before this global health burden is alleviated and HCV is eradicated. Meanwhile, the mankind is struggling to achieve this without a prophylactic vaccine. Reduced drug cost, improved access to medication, and most importantly, treatment uptake is also pivotal in combating this disease. To avoid end-stage liver diseases, liver cancer, and the need for liver transplantation, this infection must be managed first with antiviral therapy and then with a prophylactic vaccine when available.

Limitations of the review

This review covers majority of the aspects relevant to the HCV genome, worldwide prevalence, transmission, risk factors, symptoms, screening methods, prevention, and treatment. Though this review describes HCV infection in a detailed manner but limitations do exist. It only covered 150 research articles and in-depth molecular aspects of genome are not covered. As an example, only Pakistan scenario is discussed and other countries are not given due weightage due to limitation of time and length restriction of the article. This article also lacks in providing information related to the artificial intelligence of HCV diagnostic methods. It also focuses more on treatment with interferon-free DAAs and do not include information on stem-cell therapy for last stage liver failure and recent advances in development of prophylactic vaccine for HCV.

Author contributions

S.A. developed the concept of the study; S.A., M.T., A.Az., M.S., M.N., M.R., A.B.S., M.Z.N., S.H., and A.J. wrote the initial draft; A.A. reviewed and corrected the main article; and A.A. and S.A. provided supervision. All the authors approved the final version of the article.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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