SAGE Open Medicine
Volume 10: 1–14
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/20503121221105957
journals.sagepub.com/home/smo
SAGE

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

Mehlayl Tariq[1], Abu Bakar Shoukat[1], Sedrah Akbar[1], Samaia Hameed[1], Muniba Zainab Naqvi[1], Ayesha Azher[1], Muhammad Saad[1,2], Muhammad Rizwan[1], Muhammad Nadeem[1], Anum Javed[1], Asad Ali[3] and Shahid Aziz[1,2]

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

[1]Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[2]BreathMAT Lab, IAD, Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad, Pakistan
[3]Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Punjab, Pakistan

Corresponding authors:
Asad Ali, Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-i-Azam Campus, Lahore, Punjab 54590, Pakistan.
Email: asad869@gmail.com

Shahid Aziz, Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan.
Emails: honeybunt_kadarmana@hotmail.com; saziz@bs.qau.edu.pk

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

burden of HCV infection can be assessed after the climax of HCV diagnostic practices.[4]

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.[4,10,11]

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.[12] 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 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 symptoms 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 virus[29] 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.[43] 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.[48,49] 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.[11] Genotypes 1, 2, and 3 are leading causes of 90% of all the infections in Europe.[11,50] 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 predominately 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.[36] 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.[51] Furthermore, genotype 4 is mainly present in Africa and Middle East but the subtype 4d was at first conceded in a Danish patient.[36,52] 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.[53] 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.[54] Genotype 3 is the most arduous to be treated for novel-IFN free DAA based therapies.[55,56]

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.[57,58] 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.[58] 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).[59,60] 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%).[61] Contrarily, Baluchistan and Khyber-Pakhtunkhwa are having less frequency of nearly 1% each.[58] 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.[62] 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.[63] Pakistan is having highest rate of injection usage worldwide with the disposal rate of 5–13 injections per capita annually.[58,64]

Syringes in Pakistan are quite inexpensive, that is, nearly Rs. 2–7 (US$0.02–$0.07) per syringe.[64] There exists no intimation 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.[65] 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.[66–68] 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)[69] 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%.[70] 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.[71] 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.[72] 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.[73] E2 operates as a chaperon for E1, therefore, when E2 is unavailable then E1 makes misfolded clusters.[74]

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.[75,76] 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.[77] Second, to activate the translation, several cell features bind to the IRES including polypyrimidine-tract-binding (PTB) protein,[78] the La antigen,[79] heterogeneous nuclear ribonucleoprotein L[80] 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.[81–84] 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.[85] 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.[86]

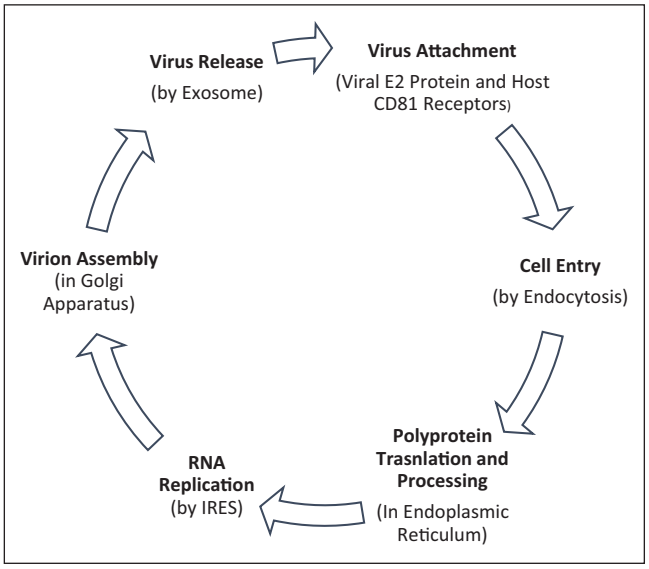

Figure 1. Graphical depiction of HCV life cycle.

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.[87] NS5B can produce RNA primer due to the elevated concentrations of the GTP or ATP.[88] Full-length genome of HCV is replicated by NS5B in vitro.[89] 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).[90–92] Glyceraldehyde-3-phosphate dehydrogenase (G3P dehydrogenase), interacts with the poly (U)-sequence in the 3′ NTR[93] and the p87 and p130 cellular proteins. HCV replication might also be obstructed by proteins from other viruses,[94] 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.[95] The nucleocapsids of the virus get their envelops 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.[96]

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.[97] 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.[98]

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.[99] 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).[100]

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.[101] 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.[102]

Cirrhosis

LC is the development of a censorious period during chronic liver disease caused by HCV. Without the provision of antiviral therapy, nearly 67%–91% of the patients have to face death because of other liver disorders such as liver function failure and liver cancer.[103] 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.[104]

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.[105] 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.[106] In last 15 years, there is an increase in the mortality rate linked with HCC.[105] 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.[127] 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.[128] 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.[129]

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.[130] 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.[131] Among medical care items which can be recycled, therapeutic injections are the second highest.[124]

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.[132,133] In regard to a study, IDUs are 46 times more vulnerable to be HCV-positive than healthcare personnel.[134]

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.[135–138] For example, patients with thalassemia and hemodialysis are at greater risk.[139]

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.[133,138] Homosexuality is also substantially associated with the elevation of the disease.[123] Extramarital affairs can also be one of the risk factors for viral hepatitis.[126]

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.[126] 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.[140]

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.[137] Dental surgeries also involve procedures, for instance, use of unsterilized tools that can aid in disease spread.[138,141] 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.[142,143]

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.[134,137]

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.[133,144] Caesarian operation is an anticipated risk.[145] 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.[146] 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

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

symptoms. 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 angioneurotic 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.[147–149]

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 up 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

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

Genotype	Drugs	Weeks
1a	Ledipasvir/sofosbuvir	8–12 weeks
1b	Elbasvir/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]

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 s 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.

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 pricking, 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, of 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 article 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., S.Ak., 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.

ORCID iDs

Sedrah Akbar https://orcid.org/0000-0001-7502-6879
Asad Ali https://orcid.org/0000-0003-1034-5873

References

1. Alter MJ, Margolis HS, Krawczynski K, et al. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *N Engl J Med* 1992; 327: 1899–1905.
2. Williams IT, Bell BP, Kuhnert W, et al. Incidence and transmission patterns of acute hepatitis C in the United States, 1982-2006. *Arch Intern Med* 2011; 171: 242–248.
3. Feinstone SM, Kapikian AZ, Purcell RH, et al. Transfusion-associated hepatitis not due to viral hepatitis type A or B. *N Engl J Med* 1975; 292: 767–770.
4. Gower E, Estes C, Blach S, et al. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; 61(Suppl. 1): S45–S57.
5. Hajarizadeh B, Grebely J and Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; 10: 553–562.
6. Leone N and Rizzetto M. Natural history of hepatitis C virus infection: from chronic hepatitis to cirrhosis, to hepatocellular carcinoma. *Minerva Gastroenterol Dietol* 2005; 51(1): 31–46.
7. Purcell RH, Walsh JH, Holland PV, et al. Seroepidemiological studies of transfusion-associated hepatitis. *J Infect Dis* 1971; 123: 406–413.
8. Wang LY, You SL, Lu SN, et al. Risk of hepatocellular carcinoma and habits of alcohol drinking, betel quid chewing and cigarette smoking: a cohort of 2416 HBsAg-seropositive and 9421 HBsAg-seronegative male residents in Taiwan. *Cancer Cause Control* 2003; 14(3): 241–250.
9. Westbrook RH and Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; 61: S58–S68.
10. Ferrarese A, Zanetto A, Gambato M, et al. Liver transplantation for viral hepatitis in 2015. *World J Gastroenterol* 2016; 22: 1570–1581.
11. Messina JP, Humphreys I, Flaxman A, et al. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; 61: 77–87.
12. Idrees M, Lal A, Malik FA, et al. Occult hepatitis C virus infection and associated predictive factors: the Pakistan experience. *Infect Genet Evol* 2011; 11: 442–445.
13. Agnello V, Chung RT and Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; 327: 1490–1495.
14. Agnello V and Elfahal M. Cryoglobulin types and rheumatoid factors associated with clinical manifestations in patients with hepatitis C virus infection. *Dig Liver Dis* 2007; 39(Suppl. 1): S25–S31.
15. Nocente R, Ceccanti M, Bertazzoni G, et al. HCV infection and extrahepatic manifestations. *Hepatogastroenterology* 2003; 50: 1149–1154.
16. Poynard T, Yuen MF, Ratziu V, et al. Viral hepatitis C. *Lancet* 2003; 362: 2095–2100.
17. Ramos-Casals M, Loustaud-Ratti V, De Vita S, et al. Sjogren syndrome associated with hepatitis C virus: a multicenter analysis of 137 cases. *Medicine* 2005; 84: 81–89.
18. Cacoub P, Poynard T, Ghillani P, et al. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999; 42(10): 2204–2212.

19. Lee MH, Yang HI, Lu SN, et al. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *J Infect Dis* 2012; 206: 469–477.
20. Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; 13: 2436–2441.
21. Spengler U. Direct antiviral agents (DAAs) —a new age in the treatment of hepatitis C virus infection. *Pharmacol Ther* 2018; 183: 118–126.
22. Heller T and Rehermann B. Acute hepatitis C: a multifaceted disease. *Semin Liver Dis* 2005; 25: 7–17.
23. Bertrand RHC, Bertrand ALX, Gomes TM, et al. An eye on hepatitis C: a review. *Arq Bras Oftalmol* 2019; 82(2): 161–167.
24. Choo QL, Richman KH, Han JH, et al. Genetic organization and diversity of the hepatitis C virus. *Proc Natl Acad Sci U S A* 1991; 88: 2451–2455.
25. Kolykhalov AA, Feinstone SM and Rice CM. Identification of a highly conserved sequence element at the 3' terminus of hepatitis C virus genome RNA. *J Virol* 1996; 70: 3363–3371.
26. Tanaka T, Kato N, Cho MJ, et al. A novel sequence found at the 3' terminus of hepatitis C virus genome. *Biochem Biophys Res Commun* 1995; 215: 744–749.
27. Gottwein JM and Bukh J. Cutting the gordian knot-development and biological relevance of hepatitis C virus cell culture systems. *Adv Virus Res* 2008; 71: 51–133.
28. Stapleton JT, Foung S, Muerhoff AS, et al. The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family Flaviviridae. *J Gen Virol* 2011; 92(Pt. 2): 233–246.
29. King AM, Lefkowitz E, Adams MJ, et al. *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*. Amsterdam: Elsevier, 2011.
30. Wieland S, Makowska Z, Campana B, et al. Simultaneous detection of hepatitis C virus and interferon stimulated gene expression in infected human liver. *Hepatology* 2014; 59(6): 2121–2130.
31. Jopling CL, Yi M, Lancaster AM, et al. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005; 309: 1577–1581.
32. Douam F, Lavillette D and Cosset FL. The mechanism of HCV entry into host cells. *Prog Mol Biol Transl Sci* 2015; 129: 63–107.
33. Calattini S, Fusil F, Mancip J, et al. Functional and biochemical characterization of hepatitis C virus (HCV) particles produced in a humanized liver mouse model. *J Biol Chem* 2015; 290: 23173–23187.
34. Gastaminza P, Kapadia SB and Chisari FV. Differential biophysical properties of infectious intracellular and secreted hepatitis C virus particles. *J Virol* 2006; 80(22): 11074–11081.
35. Bukh J, Miller RH and Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. *Semin Liver Dis* 1995; 15: 41–63.
36. Bukh J, Purcell RH and Miller RH. At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. *Proc Natl Acad Sci U S A* 1993; 90: 8234–8238.
37. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; 74(Pt. 11): 2391–2399.
38. Kretzer IF, do Livramento A, da Cunha J, et al. Hepatitis C worldwide and in Brazil: silent epidemic–data on disease including incidence, transmission, prevention, and treatment. *Sci World J* 2014; 2014: 827849.
39. Bukh J, Apgar CL, Engle R, et al. Experimental infection of chimpanzees with hepatitis C virus of genotype 5a: genetic analysis of the virus and generation of a standardized challenge pool. *J Infect Dis* 1998; 178(4): 1193–1197.
40. Chamberlain RW, Adams NJ, Taylor LA, et al. The complete coding sequence of hepatitis C virus genotype 5a, the predominant genotype in South Africa. *Biochem Biophys Res Commun* 1997; 236: 44–49.
41. Robertson B, Myers G, Howard C, et al. Classification, nomenclature, and database development for hepatitis C virus (HCV) and related viruses: proposals for standardization. International Committee on Virus Taxonomy. *Arch Virol* 1998; 143(12): 2493–2503.
42. Simmonds P, Bukh J, Combet C, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* 2005; 42(4): 962–973.
43. Murphy DG, Sablon E, Chamberland J, et al. Hepatitis C virus genotype 7, a new genotype originating from central Africa. *J Clin Microbiol* 2015; 53(3): 967–972.
44. Smith DB, Bukh J, Kuiken C, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* 2014; 59: 318–327. DOI: 10.1002/hep.26744.
45. Li C, Lu L, Murphy DG, et al. Origin of hepatitis C virus genotype 3 in Africa as estimated through an evolutionary analysis of the full-length genomes of nine subtypes, including the newly sequenced 3d and 3e. *J Gen Virol* 2014; 95(Pt. 8): 1677–1688.
46. Lu L, Xu Y, Yuan J, et al. The full-length genome sequences of nine HCV genotype 4 variants representing a new subtype 4s and eight unclassified lineages. *Virology* 2015; 482: 111–116.
47. Simmonds P. The origin of hepatitis C virus. *Curr Top Microbiol Immunol* 2013; 369: 1–15.
48. Galli A and Bukh J. Comparative analysis of the molecular mechanisms of recombination in hepatitis C virus. *Trends Microbiol* 2014; 22(6): 354–364.
49. Kalinina O, Norder H, Mukomolov S, et al. A natural intergenotypic recombinant of hepatitis C virus identified in St. Petersburg. *J Virol* 2002; 76(8): 4034–4043.
50. Esteban JI, Sauleda S and Quer J. The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008; 48(1): 148–162.
51. Corbet S, Bukh J, Heinsen A, et al. Hepatitis C virus subtyping by a core-envelope 1-based reverse transcriptase PCR assay with sequencing and its use in determining subtype distribution among Danish patients. *J Clin Microbiol* 2003; 41(3): 1091–1100.
52. Bukh J, Purcell RH and Miller RH. Sequence analysis of the core gene of 14 hepatitis C virus genotypes. *Proc Natl Acad Sci U S A* 1994; 91: 8239–8243.
53. Negro F. Mechanisms and significance of liver steatosis in hepatitis C virus infection. *World J Gastroenterol* 2006; 12: 6756–6765.
54. Pawlotsky JM, Feld JJ, Zeuzem S, et al. From non-A, non-B hepatitis to hepatitis C virus cure. *J Hepatol* 2015; 62(Suppl. 1): S87–S99.

55. Goossens N and Negro F. Is genotype 3 of the hepatitis C virus the new villain? *Hepatology* 2014; 59(6): 2403–2412.
56. Pawlotsky JM. New hepatitis C therapies: the toolbox, strategies, and challenges. *Gastroenterology* 2014; 146(5): 1176–1192.
57. Janjua NZ. Injection practices and sharp waste disposal by general practitioners of Murree, Pakistan. *J Pak Med Assoc* 2003; 53(3): 107–111.
58. Qureshi H, Bile KM, Jooma R, et al. Prevalence of hepatitis B and C viral infections in Pakistan: findings of a national survey appealing for effective prevention and control measures. *East Mediterr Health J* 2010; 16 Suppl: S15–S23. DOI: 10.26719/2010.16.SUPP.15.
59. Merat S and Poustchi H. Hepatitis C in Iran. How extensive of a problem is it? *Arch Iran Med* 2012; 15(5): 268.
60. Sibley A, Han KH, Abourached A, et al. The present and future disease burden of hepatitis C virus infections with today's treatment paradigm—volume 3. *J Viral Hepat* 2015; 22(Suppl. 4): 21–41.
61. Karamat K. Problem, prevalence and prevention of viral hepatitis in Pakistan, 2007, https://www.yumpu.com/en/document/read/7458542/problem-prevalence-and-prevention-of-viral-hepatitis-in-pakistan-/12
62. Umer M and Iqbal M. Hepatitis C virus prevalence and genotype distribution in Pakistan: comprehensive review of recent data. *World J Gastroenterol* 2016; 22: 1684–1700.
63. Akbar H, Idrees M, Manzoor S, et al. Hepatitis C virus infection: a review of the current and future aspects and concerns in Pakistan. *J Gen Mol Virol* 2009; 1: 12–18.
64. Khan AA, Saleem M, Qureshi H, et al. Comparison of need and supply of syringes for therapeutic injections in Pakistan. *J Pak Med Assoc* 2012; 62(11): 1149–1153.
65. Abbas M, Hussain MF, Raza S, et al. Frequency and awareness of hepatitis B and C in visitors of Hepatitis Awareness Mela. *J Pak Med Assoc* 2010; 60(12): 1069–1071.healthcare
66. Aslam M, Taj T, Ali A, et al. Needle stick injuries among healthcare workers of public sector tertiary care hospitals of Karachi. *J Coll Physicians Surg Pak* 2010; 20(3): 150–153.
67. Khan AJ, Luby SP, Fikree F, et al. Unsafe injections and the transmission of hepatitis B and C in a periurban community in Pakistan. *Bull World Health Organ* 2000; 78(8): 956–963.
68. Yakoob J, Jafri W, Siddiqui S, et al. Dengue fever with hepatitis E and hepatitis A infection. *J Pak Med Assoc* 2009; 59: 176–177.
69. World Health Organization. *World health statistics 2016: monitoring health for the SDGs, sustainable development goals*. Geneva: World Health Organization, 2016.
70. Blight K and Gowans E. In situ hybridization and immunohistochemical staining of hepatitis C virus products. *Viral Hepatitis Rev* 1995; 1: 143–155.
71. Neumann AU, Lam NP, Dahari H, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; 282: 103–107.
72. Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. *Science* 1998; 282: 938–941.
73. Flint M, Maidens C, Loomis-Price LD, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. *J Virol* 1999; 73(8): 6235–6244.
74. Michalak JP, Wychowski C, Choukhi A, et al. Characterization of truncated forms of hepatitis C virus glycoproteins. *J Gen Virol* 1997; 78(Pt. 9): 2299–2306.
75. Tsukiyama-Kohara K, Iizuka N, Kohara M, et al. Internal ribosome entry site within hepatitis C virus RNA. *J Virol* 1992; 66: 1476–1483.
76. Wang C, Sarnow P and Siddiqui A. Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. *J Virol* 1993; 67(6): 3338–3344.
77. Ito T, Tahara SM and Lai MM. The 3'-untranslated region of hepatitis C virus RNA enhances translation from an internal ribosomal entry site. *J Virol* 1998; 72(11): 8789–8796.
78. Ali N and Siddiqui A. Interaction of polypyrimidine tract-binding protein with the 5' noncoding region of the hepatitis C virus RNA genome and its functional requirement in internal initiation of translation. *J Virol* 1995; 69: 6367–6375.
79. Ali N and Siddiqui A. The La antigen binds 5' noncoding region of the hepatitis C virus RNA in the context of the initiator AUG codon and stimulates internal ribosome entry site-mediated translation. *Proc Natl Acad Sci U S A* 1997; 94: 2249–2254.
80. Hahm B, Kim YK, Kim JH, et al. Heterogeneous nuclear ribonucleoprotein L interacts with the 3' border of the internal ribosomal entry site of hepatitis C virus. *J Virol* 1998; 72(11): 8782–8788.
81. Grakoui A, Wychowski C, Lin C, et al. Expression and identification of hepatitis C virus polyprotein cleavage products. *J Virol* 1993; 67(3): 1385–1395.
82. Hijikata M, Kato N, Ootsuyama Y, et al. Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. *Proc Natl Acad Sci U S A* 1991; 88: 5547–5551.
83. Lin HH, Kao JH, Hsu HY, et al. Possible role of high-titer maternal viremia in perinatal transmission of hepatitis C virus. *J Infect Dis* 1994; 169(3): 638–641.
84. Mizushima H, Hijikata M, Asabe S, et al. Two hepatitis C virus glycoprotein E2 products with different C termini. *J Virol* 1994; 68(10): 6215–6222.
85. Wu Z, Yao N, Le HV, et al. Mechanism of autoproteolysis at the NS2-NS3 junction of the hepatitis C virus polyprotein. *Trends Biochem Sci* 1998; 23(3): 92–94.
86. Love RA, Parge HE, Wickersham JA, et al. The crystal structure of hepatitis C virus NS3 proteinase reveals a trypsin-like fold and a structural zinc binding site. *Cell* 1996; 87: 331–342.
87. Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* 1998; 36(4): 862–865.
88. Luo G, Hamatake RK, Mathis DM, et al. De novo initiation of RNA synthesis by the RNA-dependent RNA polymerase (NS5B) of hepatitis C virus. *J Virol* 2000; 74(2): 851–863.
89. Lohmann V, Körner F, Herian U, et al. Biochemical properties of hepatitis C virus NS5B RNA-dependent RNA polymerase and identification of amino acid sequence motifs essential for enzymatic activity. *J Virol* 1997; 71(11): 8416–8428.
90. Chung RT and Kaplan LM. Heterogeneous nuclear ribonucleoprotein I (hnRNP-I/PTB) selectively binds the conserved 3' terminus of hepatitis C viral RNA. *Biochem Biophys Res Commun* 1999; 254: 351–362.
91. Ito T and Lai MM. Determination of the secondary structure of and cellular protein binding to the 3'-untranslated region of the hepatitis C virus RNA genome. *J Virol* 1997; 71(11): 8698–8706.

92. Tsuchihara K, Tanaka T, Hijikata M, et al. Specific interaction of polypyrimidine tract-binding protein with the extreme 3'-terminal structure of the hepatitis C virus genome, the 3'X. *J Virol* 1997; 71(9): 6720–6726.
93. Petrik J, Parker H and Alexander GJM. Human hepatic glyceraldehyde-3-phosphate dehydrogenase binds to the poly(U) tract of the 3' non-coding region of hepatitis C virus genomic RNA. *J Gen Virol* 1999; 80(Pt. 12): 3109–3113.
94. Sugawara Y, Makuuchi M, Kato N, et al. Enhancement of hepatitis C virus replication by Epstein-Barr virus-encoded nuclear antigen 1. *EMBO J* 1999; 18: 5755–5760.
95. Shimoike T, Mimori S, Tani H, et al. Interaction of hepatitis C virus core protein with viral sense RNA and suppression of its translation. *J Virol* 1999; 73: 9718–9725.
96. Sato Y, Nakata K, Kato Y, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993; 328: 1802–1806.
97. Mirandola S, Bowman D, Hussain MM, et al. Hepatic steatosis in hepatitis C is a storage disease due to HCV interaction with microsomal triglyceride transfer protein (MTP). *Nutr Metab* 2010; 7: 13.
98. Stevenson HL and Utay NS. Hepatic steatosis in HCV-infected persons in the direct-acting antiviral era. *Trop Dis Travel Med Vaccines* 2016; 2: 21.
99. Kralj D, Virović Jukić L, Stojsavljevic S, et al. Hepatitis C virus, insulin resistance, and steatosis. *J Clin Transl Hepatol* 2016; 4: 66–75.
100. Esfeh JM and Ansari-Gilani K. Steatosis and hepatitis C. *Gastroenterol Rep* 2016; 4: 24–29.
101. Khatun M and Ray RB. Mechanisms underlying hepatitis C virus-associated hepatic fibrosis. *Cells* 2019; 8: 1249.
102. Ehsan N, Sweed D and Elsabaawy M. Evaluation of HCV-related liver fibrosis post-successful DAA therapy. *Egypt Liver J* 2021; 11: 1–7.
103. Toshikuni N, Arisawa T and Tsutsumi M. Hepatitis C—related liver cirrhosis—strategies for the prevention of hepatic decompensation, hepatocarcinogenesis, and mortality. *World J Gastroenterol* 2014; 20: 2876–2887.
104. Ullah A, Rehman IU, Ahmad J, et al. Hepatitis-C virus and cirrhosis: an overview from Khyber Pakhtunkhwa province of Pakistan. *Viral Immunol* 2020; 33(5): 396–403.
105. Singh AK, Kumar R and Pandey AK. Hepatocellular carcinoma: causes, mechanism of progression and biomarkers. *Curr Chem Genom Transl Med* 2018; 12: 9–26.
106. Balogh J, Victor D 3rd, Asham EH, et al. Hepatocellular carcinoma: a review. *J Hepatocell Carcinoma* 2016; 3: 41–53.
107. Large MK, Kittlesen DJ and Hahn YS. Suppression of host immune response by the core protein of hepatitis C virus: possible implications for hepatitis C virus persistence. *J Immunol* 1999; 162: 931–938.
108. Taylor DR, Shi ST, Romano PR, et al. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 1999; 285: 107–110.
109. Gale M Jr, Kwieciszewski B, Dossett M, et al. Antiapoptotic and oncogenic potentials of hepatitis C virus are linked to interferon resistance by viral repression of the PKR protein kinase. *J Virol* 1999; 73: 6506–6516.
110. Saraceni C and Birk J. A review of hepatitis B virus and hepatitis C virus immunopathogenesis. *J Clin Transl Hepatol* 2021; 9: 409–418.
111. Khayrulla F, Mukhaye A, Zaynitdin K, et al. Immunological aspects of the pathogenesis of chronic HCV infection. *Ann Roman Soc Cell Biol* 2021; 2021: 4164–4173.
112. Salama SAMMA. The interplay between endogenous inflammatory mediators and microbial components in innate immunity, 2021, https://limo.libis.be/primo-explore/fulldisplay?docid=LIRIAS3367295&context=L&vid=Lirias&search_scope=Lirias&tab=default_tab&fromSitemap=1
113. Lee J and Ou JJ. Hepatitis C virus and intracellular antiviral response. *Curr Opin Virol* 2022; 52: 244–249.
114. Thimme R. T cell immunity to hepatitis C virus: lessons for a prophylactic vaccine. *J Hepatol* 2021; 74(1): 220–229.
115. Zhao HJ, Hu YF, Han QJ, et al. Innate and adaptive immune escape mechanisms of hepatitis B virus. *World J Gastroenterol* 2022; 28: 881–896.
116. Ribeiro CRA, Beghini DG, Lemos AS, et al. Cytokines profile in patients with acute and chronic hepatitis B infection. *Microbiol Immunol* 2022; 66(1): 31–39.
117. Liberto MC and Marascio N. *Special Issue "Chronic HCV infection: clinical advances and eradication perspectives."* Basel: Multidisciplinary Digital Publishing Institute, 2022, p. 359.
118. Tai AW and Vargas HE. *Chronic hepatitis C*. Hoboken, NJ: Wiley, 2022, pp. 1861–1877.
119. Holub M, Stráníková A, Beran O, et al. Chronic hepatitis C virus infection modulates the transcriptional profiles of CD4+ T cells. *Can J Infect Dis Med Microbiol* 2021; 2021: 6689834.
120. Nguyen LN, Nguyen LNT, Zhao J, et al. Immune activation induces telomeric DNA damage and promotes short-lived effector T cell differentiation in chronic HCV infection. *Hepatology* 2021; 74: 2380–2394.
121. Lu X, Song B, Weng W, et al. CD8+ stem cell-like memory T cell subset is associated with disease progression in chronic hepatitis C virus infection, 2022, https://assets.researchsquare.com/files/rs-1533048/v1/1f63f3fd-a240-4519-b94e-7ec66e7ceecb.pdf?c=1652357998
122. Hensel N, Gu Z, Sagar, et al. Memory-like HCV-specific CD8+ T cells retain a molecular scar after cure of chronic HCV infection. *Nat Immunol* 2021; 22: 229–239.
123. Khan NU, Ali I, Ahmad NU, et al. Prevalence of active HCV infection among the blood donors of Khyber Pakhtunkwa and FATA region of Pakistan and evaluation of the screening tests for anti-HCV. *Virol J* 2011; 8: 1–3.
124. Asad M, Ahmed F, Zafar H, et al. Frequency and determinants of hepatitis B and C virus in general population of Farash Town, Islamabad. *Pak J Med Sci* 2015; 31(6): 1394–1398.
125. Khan A, Tareen AM, Ikram A, et al. Prevalence of HCV among the young male blood donors of Quetta region of Balochistan, Pakistan. *Virol J* 2013; 10: 1–4.
126. Memon AR, Shafique K, Memon A, et al. Hepatitis B and C prevalence among the high risk groups of Pakistani population. A cross sectional study. *Arch Public Health* 2012; 70: 9.
127. Awan ZUR, Shah AH, Khan S, et al. Molecular prevalence of hepatitis B virus infection in Khyber Pakhtunkhwa, Pakistan. *Int J Med Med Sci* 2012; 4: 123–127.
128. Safi SZ, Badshah Y, Waheed Y, et al. Distribution of hepatitis C virus genotypes, hepatic steatosis and their correlation with clinical and virological factors in Pakistan. *Asian Biomed* 2010; 4: 253–262.

129. Shah HB, Dar MK, Jamil AA, et al. Knowledge, attitudes and practices of hepatitis B and C among barbers of urban and rural areas of Rawalpindi and Islamabad. *J Ayub Med Coll Abbottabad* 2015; 27(4): 832–836.
130. Pozzetto B, Memmi M, Garraud O, et al. Healthcare-associated hepatitis C virus infection. *World J Gastroenterol* 2014; 20: 17265–17278.
131. Janjua NZ and Nizamy MA. Knowledge and practices of barbers about hepatitis B and C transmission in Rawalpindi and Islamabad. *J Pak Med Assoc* 2004; 54(3): 116–119.
132. Idrees M. Development of an improved genotyping assay for the detection of hepatitis C virus genotypes and subtypes in Pakistan. *J Virol Methods* 2008; 150(1–2): 50–56.
133. Safi SZ, Waheed Y, Sadat J, et al. Molecular study of HCV detection, genotypes and their routes of transmission in North West Frontier Province, Pakistan. *Asian Pac J Trop Biomed* 2012; 2(7): 532–536.
134. Ahmed F, Irving WL, Anwar M, et al. Prevalence and risk factors for hepatitis C virus infection in Kech District, Balochistan, Pakistan: most infections remain unexplained. A cross-sectional study. *Epidemiol Infect* 2012; 140(4): 716–723.
135. Akhtar N, Bilal M, Rizwan M, et al. Genotypes of hepatitis C virus in relapsed and non-respondent patients and their response to anti-viral therapy in district Mardan, Khyber Pakhtunkhawa, Pakistan. *Asian Pac J Cancer Prev* 2015; 16: 1037–1040.
136. Ghias M and Pervaiz MK. Identification of epidemiological risk factors for hepatitis C in Punjab, Pakistan. *J Ayub Med Coll Abbottabad* 2009; 21(2): 156–161.
137. Qazi HA, Saleem K, Mujtaba I, et al. Prevalence and factors associated with HCV (hepatitis C virus) seropositivity in Islamabad, Pakistan. *Acta Med Iran* 2010; 48(6): 394–398.
138. Shafiq MI, Gauhar A, Akram M, et al. Thyroid peroxidase antibodies in non-interferon treated hepatitis C patients in Pakistan. *Biomed Res Int* 2015; 2015: 172981.
139. Ali I, Siddique L, Rehman LU, et al. Prevalence of HCV among the high risk groups in Khyber Pakhtunkhwa. *Virol J* 2011; 8: 1–4.
140. Majid A, Khan MS and Ullah S. Rising prevalence of hepatitis B and C and risk factors at district headquarter teaching hospital Bannu, Khyber Pakhtunkhwa. *J Coll Physicians Surg Pak* 2010; 20(7): 492–493.
141. Fayyaz M, Ghous SM, Abbas I, et al. Frequency of hepatitis B and C in patients seeking treatment at the dental section of a tertiary care hospital. *J Ayub Med Coll Abbottabad* 2015; 27(2): 395–397.
142. Abbasi A, Bhutto AR, Butt N, et al. HDV seroprevalence in HBsAg positive patients. *J Coll Physicians Surg Pak* 2014; 24: 624–627.
143. Bibi S, Dars S, Ashfaq S, et al. Seroprevalence and risk factors for hepatitis C virus (HCV) infection in pregnant women attending public sector tertiary care hospital in Hyderabad Sindh. *Pak J Med Sci* 2013; 29(2): 505–508.
144. Jiwani N and Gul RB. A silent storm: hepatitis C in Pakistan. *J Pioneer Med Sci* 2011; 1: 89–91.
145. Rauf A, Nadeem MS, Ali A, et al. Prevalence of hepatitis B and C in internally displaced persons of war against terrorism in Swat, Pakistan. *Eur J Public Health* 2011; 21(5): 638–642.
146. Quddus A, Luby SP, Jamal Z, et al. Prevalence of hepatitis B among Afghan refugees living in Balochistan, Pakistan. *Int J Infect Dis* 2006; 10(3): 242–247.
147. Behzadifar M, Gorji HA, Rezapour A, et al. Comparison of prevention, screening and treatment of hepatitis C in Iran, Egypt and Georgia. *J Virus Erad* 2019; 5: 116–121.
148. Naseer K, Saleem M, Ali S, et al. Identification of new spectral signatures from hepatitis C virus infected human sera. *Spectrochim Acta A Mol Biomol Spectrosc* 2019; 222: 117181.
149. Sakamaki A, Kamimura K, Fukui N, et al. A case report of psychiatric symptoms following direct-acting antiviral and ribavirin combination therapy for chronic hepatitis C in a patient with innate anxiety. *BMC Gastroenterol* 2019; 19: 85.
150. Wlassow M, Poiteau L, Roudot-Thoraval F, et al. The new Xpert HCV viral load real-time PCR assay accurately quantifies hepatitis C virus RNA in serum and whole-blood specimens. *J Clin Virol* 2019; 117: 80–84.
151. European Association for Study of Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; 60: 392–420.
152. European Association for Study of Liver. EASL recommendations on treatment of hepatitis C 2018. *J Hepatol* 2018; 69: 461–511.
153. Shah H, Bilodeau M, Burak KW, et al. The management of chronic hepatitis C: 2018 guideline update from the Canadian Association for the Study of the Liver. *CMAJ* 2018; 190: E677–E687.
154. Bell AM, Wagner JL, Barber KE, et al. Elbasvir/Grazoprevir: a review of the latest agent in the fight against Hepatitis C. *Int J Hepatol* 2016; 2016: 3852126. DOI: 10.1155/2016/3852126.