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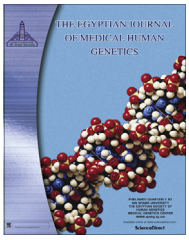

REVIEW

An overview on hepatitis C virus genotypes and its control

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KEYWORDS

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Abstract Hepatitis C virus (HCV) is a blood borne, circular and positive single stranded virus with high spread rates. With the passage of time the frequency of HCV is increasing in different parts of the world. HCV is a major cause, which may end in liver cirrhosis and hepatocellular carcinoma. HCV has six main genotypes with many subtypes, which have variable sequence homology with each other. Symptoms can appear anytime from 2 weeks to 6 months, which include jaundice, fatigue, gray-colored stool, joint pain, belly pain, weakness, anorexia, itchy skin and dark urine. Genotyping is more significant for planning of HCV treatment period and helps to cure HCV infections. For the quantification and identification of hepatitis C virus-ribonucleic acid, many molecular techniques are performed; the most significant are HCV ELISA, quantitative HCV-RNA PCR and recombinant immunoblot assay. PCR is the major technique targeting 5′ untranslated region (UTR). HCV can be transmitted by contaminated blood, ear and nose piercing and contaminated medical instruments. To overcome the rate of HCV, guidance should be provided to make aware the persons about risk factors, transmission and prevention. Discovery and designing of new therapies and vaccines to overcome this disease are the necessity of the present era. Four types of vaccines such as vector vaccines, peptide vaccines, DNA vaccines and recombinant protein vaccines are available in clinical trials.

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Contents

1. History and structure of hepatitis C virus

Hepatitis C virus (HCV) is an infectious pathogen causing great damage to the liver [1]. HCV was initially isolated from the serum of an individual with non-A, non-B hepatitis discovered by Choo et al. in 1989 [2]. Briefly after cloning of HCV, the newly discovered virus was found to be the main reason of about 90% of non-A, non-B hepatitis in the US [3]. It is the most common cause of chronic liver disease ending in liver cirrhosis and hepatocellular carcinoma [4]. Globally, it is the main cause of death and morbidity [5] with major global health issues affecting 180 million individuals of the world and 10 million people in Pakistan [6]. Every year about 3–4 million individuals are affected by HCV [7]. About 27% individuals are infected and have cirrhosis and 25% have hepatocellular carcinoma [8].

HCV is enveloped, small circular, positive-sense and single stranded ribonucleic acid (RNA) virus from genus *Hepacivirus*, family *Flaviviridae* with a diameter of 50 nm [9]. The total length of RNA genome is about 9.6 kb with one open reading frame (ORF) and 5′ and 3′ untranslated regions (UTRs) at both edges [10]. 5′UTR is a more conserved part of HCV genome, which helped in evolutionary studies and genotyping [11,12].

The open reading frame encodes a polyprotein, which is comprised of 10 viral proteins named as Core (C), E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Fig. 1). The 3 structural proteins are C, E1 and E2, while the 7 nonstructural proteins are P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B [13]. Among structural proteins, core protein (21 kDa) consists of 191 amino acids, which are important components of nucleocapsid. Core protein modulates gene transcription, cell death, cell proliferation and interference metabolism leading to oxidative stress, liver steatosis and finally hepato cellular carcinoma (HCC). HCV envelope proteins E1 and E2 are generally glycosylated and have played a major role in cell entry. Protein P7 is responsible for ion channel and virus assembly [14]. The proteins NS3 serine protease and NS5B played a

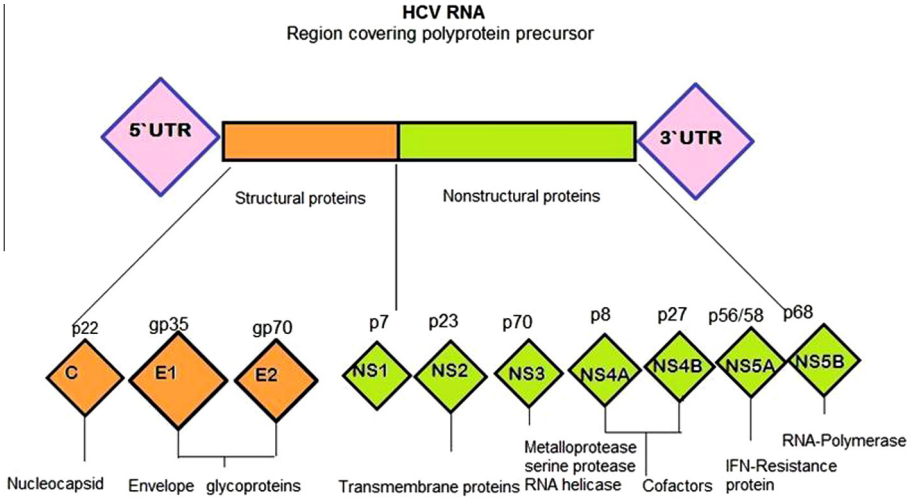

Figure 1 Proteins encoded by HCV genome. All the structural and nonstructural proteins are shown.

major role in viral replication. Main targets for antiviral drug development are structural proteins, NS5B RNA-dependent RNA polymerase and NS3 serine protease [15].

HCV has six main genotypes (1–6) with multiple subtypes. The most worldwide epidemic genotypes are 1–3. Genotyping is most significant for planning of HCV treatment and helps to cure HCV infections [16]. Due to the high prevalence and increasing rate of HCV infected persons day by day, the aim of the present review was to collect information about HCV virus, its symptoms and cure by awareness on utilization of sterilized instruments/syringes in health care centers, preparing antiviral drugs, introducing new therapies and vaccines for HCV control.

2. Frequency of HCV genotypes and infections

HCV-1, HCV-2 and HCV-3 are distributed worldwide, whereas HCV-4, HCV-5 and HCV-6 are present in certain areas of the globe. The frequency of HCV is highest in few countries and areas, such as in Europe 25 million cases are HCV positive [17]. In Pakistan, HCV is 5% prevalent [18], whereas 5–9% prevalence is detected in Khyber Pakhtunkhwa province of Pakistan [19] causing liver cancer [20]. HCV-4 is prevalent among Middle East and Africa causing more than 80% of HCV infections and has recently spread to several European countries. Egypt has the highest prevalence of HCV worldwide (15%) and the highest frequency of HCV-4 responsible for almost 90% of infections and a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and transplantation in the country. Although HCV-4 is the cause of about 20% of the 170 million cases of hepatitis C in the world [21]. In the United States, the number of new cases of HCV infection has declined from a peak of 200,000 annually to about 17,000 in 2007. Up to 85% of newly infected people failed to eliminate the virus and become chronically infected. HCV infection is the leading cause of liver transplantation in the US and is a risk factor for liver cancer [22].

3. Acute infection and chronic infection

Acute hepatitis C infection is infrequently diagnosed as the majority of acutely infected individuals are asymptomatic. In the transfusion setting, acute HCV infection has been known to be 70–80% asymptomatic and 20–30% of adults showed clinical symptoms [23,24]. Chronic hepatitis C is marked by the persistence of HCV-RNA in the blood for at least 6 months after onset of acute infection. HCV is self-limiting in only 15–25% of patients, whom HCV-RNA in the serum becomes undetectable and ALT levels returned to normal. Approximately 75–85% of infected patients do not clear the virus by 6 months and chronic hepatitis developed in them. The rate of chronic HCV infection is affected by many factors, including the age at time of infection, gender, ethnicity and development of jaundice during acute infection [25].

4. Route of transmission and symptoms of HCV

HCV can be transmitted through intravenous drug use (IVDU), blood to blood contact, birth to an infected mother, blood products, organ transplantation, use of unsterilized injection needles, use of unsterilized instruments for nose and ear piercing or tattoos (Table 1) and these reasons are the major sources of transmission of HCV in Pakistan [26,27]. In developing countries HCV is transmitted by IVDU, sexual exposure and blood products [26,28]. Virus cannot be transmitted from one person to other by sharing drink or food with an infected individual [29].

Table 1 Different transmission routes of HCV identified in different studies.

Transmission routes	Percentage
Dental treatment	1.6
IVDU	60
Blood transfusion	10
Tattoo	46.3
Ear piercing	30.1
Needle stick	2
Sexual transmission	15
Nail trimming	24.3
Shaving	14.5
Surgical treatments	7.6

Symptoms can appear anytime from 2 weeks to 6 months after a person is infected with the virus. Symptoms include jaundice, fatigue, gray-colored stool, joint pain, belly pain, weakness, anorexia, itchy skin and dark urine. Mild cognitive problems and fatigue are the major symptoms of chronic hepatitis C [25]. A study conducted in Egypt showed the symptoms of HCV including history of fatigue, diarrhea and abdominal pains. Another study performed on 77 Spanish and Italian children revealed that HCV was generally asymptomatic among them [30].

5. Diagnosis and genotyping of HCV

For the quantification and detection of HCV-RNA, many molecular biology techniques are described (Table 2). The significant technique is PCR targeting 5′UTR region [31]. Several tests are used to diagnose the hepatitis C virus, which include HCV antibody enzyme immunoassay (ELISA), quantitative HCV-RNA PCR and recombinant immunoblot assay [32]. HCV-RNA by PCR is the quickest and most informative method for HCV detection, while long infection time is required to detect virus using antibodies [33]. The hepatitis C diagnosis mostly depends on serological assay and HCV-RNA. For the epidemiology surveillance and screening, confirmatory recombinant immunoblot assay and ELISA are performed for the identification of HCV-specific antibodies (anti-ssHCV). For the identification of viral genome and confirmation of active infection, qualitative polymerase chain reaction is used. This methodology is quite effective in monitoring disease activity and treatment response [34].

6. Bioinformatics analysis and databases for HCV

A study conducted in Saudi Arabia on HCV positive samples by sequencing their 5′UTR regions revealed the identification of new HCV isolates. For sequencing and amplification, specific primers were used which target the 5′UTR region. In this study most of the HCV isolates were G4 (76.4%) and small

Table 2 Different diagnostic techniques for HCV detection [35,36]. TMA: transcription mediated amplification, bDNA: branched DNA, RT-PCR: reverse transcription-PCR, qPCR: quantitative PCR.

Assay	Procedures	Lower limit of detection (dynamic range) IU/mL
Versant HCV-RNA	TMA (manual)	10
Cobas TaqMan HCV test	Real-time PCR (semi automated)	15 (43–69,000,000)
Amplicor HCV v2.0	RT-PCR (manual)	50
Abbott real-time	Real-time PCR (semi automated)	30 (12–100,000,000)
Amplicor HCV monitor	RT-PCR (manual)	50 (600–700,000)
Super Quant	Competitive reverse transcriptase PCR (semi automated)	30–1,470,000
Versant HCV-RNA 3.0	bDNA (semi automated)	18 (43–69,000,000)
Amplicor HCV monitor v2.0	RT-PCR (manual)	600 (600–500,000)
Cobas Ampliprep and TaqMan	qPCR (semi automated)	18 (43–69,000,000)

numbers were G1 (19.6%). Los Alamos HCV database was used for comparing the received HCV sequence with HCV reference sequences taking whole genome for phylogenetic analysis. Highly similar sequences (98–100%) were selected, where majority of the isolates showed homology with G4 reference sequence. Few isolates were identical with different subtypes (G4, G1 and G6). Phylogenetic analysis represented the similarity of most isolates, which were identical to the Egyptian, East and North American isolates. Multiple sequence alignment analysis and sequence WebLogos represented 92–95.5% nucleotide conservation of HCV isolates with G4 and G1. All the resulting sequences were submitted to the GenBank database of NCBI [37].

7. Prevalence of HCV genotypes

HCV prevalence is variable in various regions of the world and among different groups of a community [38]. Genotype 1a is most prevalent in the United States and Northern Europe (Table 1), while 1b is the most common genotype worldwide [39]. In Europe and Japan, genotype 2a and 2b are most prevalent, while 2c subtype is most frequent in Northern Italy. In Africa and Middle East, the most prevalent genotype is 4 (Table 3). Genotype 5 and 6 are most common in South Africa and Asia [40,41]. Genotype 3a is most common in Pakistan in comparison to 3b and 1a [42]. In the nucleotide composition of genome, the six main genotypes are about 30–35% different from one another, while approximately 20–25% subtypes are different from each other [39].

Genotype 1 is more common in the world including 83.4 million cases (42.6% of total HCV cases), of which about 1/3 are present in East Asia. After genotype 1, genotype 3 is more common worldwide including 54.3 million cases (30.1% of HCV cases). G-2, G-4 and G-6 are responsible for a total 22.8% of whole cases, while G-5 includes the remaining percentage (>1%). In most countries G-1 and G-3 are most prevalent in comparison to other genotypes while G-4 and G-5 are prevalent in less developed countries [44]. Hospital based studies of various Pakistani cities revealed that the prevalence rate of HCV is 5.31% (Islamabad), 2.45% (Rawalpindi), 4.0% (Multan), 20.89% (Mardan), 5% (Faisalabad), 4–6% (Buner) and 25.7% (Northern areas) [34,45]. A similar study conducted in Bangladesh revealed that G-3 was the most prevalent genotype in comparison to other genotypes [16].

A study conducted on HCV-RNA positive patients in Isfahan province (Iran) revealed that the common rate of HCV genotypes was 3a (61.2%), 1a (29.5%), 1b (5.1%), genotype 2 (2%) and mixed types 1a + 3a (2%) [46]. In other studies type 3 cases are completely represented by subtype 3a, while type 4 cases (52.3%) are represented by subtype 4c and 4d in Italy [47]. Several other studies confirmed the high prevalence of genotype 1a and 1b in HCV patients from different parts of the world [48–50].

Table 3 Worldwide prevalence of HCV genotypes.

Countries	Prevalent genotypes	Refs.
Northern Europe and United States	G1a	[10]
America, Europe, Japan and Italy	G2a, G2b, G2c	[40,41]
India, Nepal and Pakistan	G3, G3a	[42]
Africa and Middle East	G4	[40,41]
South Africa	G5	[43]
Hong Kong and Southeast Asia	G6	[43]

8. HCV treatment by different therapies, vaccines and local remedies

General treatment is recommended for HCV patients with complications in the liver (Table 4). For the treatment of chronic hepatitis C, the combination of pegylated interferon

Table 4 Treatments of HCV by pegylated interferon + ribavirin. LD RBV: lower dose of ribavirin, SD RBV: standard dose of ribavirin.

HCV Genotypes	Treatment regimen	Time (weeks)	SVR (%)	Citation
HCV-1	PegIFN + SD RBV	48	44	[57]
	PegIFN + SD RBV	24	94	[58]
	PegINF alpha-2b	48	42	[59]
HCV-2/3	Ribavirin	48	76	[60]
	PegINF + LD RBV	24	75	[57]
	PegIFN + SD RBV	16	100	[57]
HCV-4	PegINF alpha-2b	24	29	[61]
	PegIFN + SD RBV	48	68	[62]
HCV-6	PegIFN + SD RBV	48	86	[63]

alpha-2a or 2b and ribavirin may be used. The endpoint of HCV treatment is sustained virological response (SVR), defined by an undetectable HCV-RNA in serum with a sensitive assay (lower limit of detection of 10–50 IU/mL) 24 weeks after the end of treatment. Genotype 2 and 3 infected patients require 24 weeks of treatment and a low dose of ribavirin (800 mg daily). In contrast, genotype 1, 4, 5 and 6 infected individuals requires 48 weeks of treatment and a body weight based dose of ribavirin (1000–1400 mg daily) [35]. A combination of treatment is administered for a period of 24–48 weeks depending on the HCV genotype (Table 4). This treatment includes ribavirin and pegylated interferon alpha in combination [32]. Around 70% and 80% cure rates were achieved for genotype 2 and 3 respectively and 45–70% for other genotypes [51]. Recently two latest therapeutic agents called as boceprevir and telaprevir are introduced in few countries, which are protease inhibitors and control HCV [52]. The combination of telaprevir or boceprevir with pegylated interferon-alpha and ribavirin is highly beneficial in controlling genotype 1 [53].

The treatment of HCV is more effective during the first six months than once it becomes chronic. If new infection develops in a person and remained uncontrolled till 8–12 weeks, pegylated interferon is strongly recommended for a period of 24 weeks to control the virus [33]. HCV genotype 1, 4, 5 or 6 and their associated diseases are controlled by administrating a combination of Sofosbuvir with ribavirin and interferon, which was found to be 90% effective against the viral diseases [54]. In Japan during December 2013, pegylated-interferon, simeprevir (SMV) and ribavirin (RBV) triple combination therapy was used to treat this disease specially genotype 1 in clinical practice [55]. In the patients of genotype 1 (42–46%), G-2 and G-3 (76–82%), a significantly increased response was detected for polyethylenglycol (PEG)-conjugated interferon alpha in comparison to the conventional interferon alpha [56] (Table 4).

Several alternative treatments or traditional remedies are used by infected people to control HCV. These include the use of Quranic verses (Dam) and other alternative and homeopathic medicines to cure it due to high costs of conventional medicines. Around 50% individuals follow alternative medicines and 65% are using both conventional and unconventional medicines [64]. Some infected patients try herbal medicine to recover from virus such as Proanthocyandin obtained from leaves of blue berry, Rhodiolakirilowii (Regel), Maxim and laccase obtained from oyster mushroom but no affective reports are documented [65].

9. Management of HCV in Pakistan

Pakistan is a developing country with less medical facilities available for its citizens. Around 75% Pakistanis are living below poverty line and cannot afford expensive medicines and drugs. More than 80 different interferon's brands are available in Pakistan, but they are out of reach of common people due to their high costs. The "Prime Minister Program for prevention and control of hepatitis" was launched in recent years and during 2006–2008, around 20,000 patients received free interferon treatment but the treated cases were only 0.01% of the total cases. After 12 years of this program, still 3/4 patients are away to receive this treatment. For Pakistani patients, currently a combination of ribavirin and interferon alpha is recommended, which showed high viral control response [34,42].

A higher sustained virological response (SVR) rate was significantly detected in Pashtoons (69.2%) in comparison to Punjabi (45.5%), Sindhi (45.5%) and Balouchi (50%) tribes after administering INF-alpha plus ribavirin to patients. The highest SVR in patients with HCV was genotype 2 (69.7%), followed by G-3 (57.3%), while the lowest SVR was investigated in G-1 infection (24.3%) [42]. Another study comprising 190 patients from both sexes between age limits 20–68 years revealed that G-3 (48.9%) was the most prevalent genotype in comparison to G-1 (42.6%). Of the overall infected persons, the response rate was 49.6%, 68.8% and 62.4% in G-1, G-2 and G-3 respectively [66].

10. Vaccines used for HCV control

Four types of vaccines (Table 5) are available in clinical trials naming vector vaccines, peptide vaccines, recombinant protein vaccines and DNA vaccines [67]. These vaccines are formed to induce targeting envelope part of the pathogen, T-cell responses and targeting nonstructural proteins [68]. Conserved HCV core protein vaccine is used with adjuvant made up of cholesterol, saponin and phospholipid and is known as ISCOMATRIX. The vaccine is well tolerated and showed a well developed humoral response [69]. According to a Japanese study, a peptide obtained from HCV core section (C35–C44) was used in Phase I. Of the 26 infected persons, 3 responded to standard therapy and 23 were non responders to ribavirin and pegylated-interferon [70]. Adenovirus vectors are Phase I vaccines. The two adenoviral vectors are Ad6 and Adch3, displaying NS3 and NS5B proteins (Table 5) [71].

Table 5 Recombinant protein, peptide and vector vaccines to control HCV and its diseases.

Vaccine type	Structure	Phase	Individuals	Outcome	Citation
Recombinant protein vaccine	HCV core protein	I, IIa	30 healthy individuals	Good tolerated and well developed antibody response	[69]
Peptide vaccine	Peptide received from core protein (C35-C44)	I	26 infected person with chronic HCV (23 non responder and three cure able)	Good tolerated and few showed response	[70]
Vector vaccines	Adenovirus vector (Ad6 and Adch3) showing NS3 and NS5B proteins	I	36 healthy individuals	Greatly immunogenic and good tolerated	[71]
DNA vaccines	ChonVac-C plasmid NS3 and 4A plus electroporation	I, IIa	12 G-1 infected treated native individuals	Good tolerated, of 6 only 4 patients are receiving high doses	[72]

11. Side effects of IFN therapy and ribavirin

In the starting week of IFN treatment, flu like symptoms can occur. Further side effects may be arthralgia, chill, headaches, fever and myalgia. Negative effects of neuropsychiatric include severe fatigue, irritability and severe depression, which may lead to suicide attempts [73]. A major side effect of ribavirin is hemolytic anemia [59].

12. Prevention and management of HCV diseases

Although few patients are cured at initial stages but the activated virus after some time will be uncontrollable and can cause human deaths. No proper vaccine and antiviral drugs are available to control 100% HCV. Our initial focus should be on prevention including safe injection practices in hospitals and another places, safe blood supply and guidance to the injection drug abuser or intravenous drug users about the risk of HCV. Health education is necessary to minimize the rate of HCV. Special programs should be launched to make aware the public on risk factors, transmission and prevention of the disease. Safe blood test and other laboratory tests are necessary for an early diagnosis of HCV [74].

13. Conclusion

HCV is a major global health issue affecting several individuals around the globe. The prevalence of HCV genotypes varies all over the world due to virus mutation, route of infection and population analysis. Monthly or yearly blood tests should be necessary for the diagnosis of HCV. In a majority of people, HCV causing liver cirrhosis and hepatocellular carcinoma cannot be diagnosed at early stages. New therapies with fewer or no side effects should be introduced to cure the virus. Guidance plays an important role in preventing people from this infection. Awareness seminars regarding the risk factor of HCV infection should be regularly arranged and discovery of new vaccines by scientists is of utmost importance to overcome this disease.

Conflict of interest

We declare that no competing interest or conflict exists among any of the authors. All the authors agreed to submit manuscript in this journal and all ethical standards are fulfilled before submission.

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References

[1] Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Maqder LS, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000;85:5887–91.
[2] Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley Houghton M. Isolation of a cDNA clone derived from a blood borne non-A, non-B viral hepatitis genome. Science 1998;244:359–62.
[3] Laucer GM. Walker hepatitis C virus infection. N Engl J Med 2001;345:41–52.
[4] Kanda T, Yokosuka O, Omata M. Hepatitis C virus and hepatocellular carcinoma. Biology 2013;2:304–16.
[5] Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, et al. Viral hepatitis and the Global Burden of disease: a need to regroup. J Viral Hepat 2013;20:600–1.
[6] Raja NK, Janjua KA. Epidemiology of hepatitis C virus infection in Pakistan. J Microbiol Immunol Infect 2008;41:4–8.
[7] Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C virus transplantation. J Hepatol 2001;35:666–78.
[8] Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007;148:56–82.
[9] Bostan N, Mahmood T. An overview about hepatitis C: a devastating virus. Crit Rev Microbiol 2010;36:91–133.
[10] Simmonds P. Genetic diversity and evolution of hepatitis C virus 15 years on. J Gen Virol 2004;85:3173–88.
[11] Margraf RL, Erali M, Liew M, Wittwer CT. Genotyping hepatitis C virus heteroduplex mobility analysis using temperature gradient capillary electrophoresis. J Clin Microbiol 2004;42:4545–51.
[12] Fan W, Zhu W, Wei L, Wang Q, Yin L, Du S, et al. Nonstructural 5A gene variability of hepatitis C virus during a 10-year follow up. J Gastroenterol 2005;40:43–51.
[13] Scheel TK, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. Nat Med 2013;19:837–49.
[14] Krekulova L, Rehak V, Riley LW. Structure and function of hepatitis C virus proteins: 15 years later. Folia Microbiol 2006;51:665–80.
[15] De Francesco R, Tomei L, Altamura S, Summa V, Migliaccio G. Approaching a new era for hepatitis C virus therapy: inhibitor of the NS3-4A serine protease and the NS5B RNA-dependent RNA polymerase. Antiviral Res 2003;58:1–16.
[16] Islam MS, Miah MR, Roy PK, Rahman O, Siddique AB, Chowdhury J, et al. Genotypes of hepatitis C virus infection in Bangladeshi population. Mymensingh Med J 2015;24:143–51.
[17] Rockstroh J, Grint D, Bosecke C. Increase in acute hepatitis C virus (HCV) incidence across Europe: which region and patient groups are affected? J Int AIDS Soc 2012;15:18116.
[18] Afridi SQ, Zahid MN, Shabbir MZ, Hussain Z, Mukhtar N, Tipu MY, et al. Prevalence of HCV genotypes in district Mardan. Virol J 2013;10:10–90.
[19] Khan N, Akmal M, Hayat M, Umar M, Ullah A, Ahmed I, et al. Geographical distribution of hepatitis C virus genotypes in Pakistan. Hepat Mon 2014;14:e20299.
[20] Munaf A, Memon MS, Kumar P, Ahmed S, Kumar MB. Comparison of viral hepatitis-associated hepatocellular carcinoma due to HBV and HCV-cohort from liver clinics in Pakistan. Asian Pac J Cancer Prev 2014;15:7563–7.
[21] Sanaa MK, Naseer IA. Hepatitis C genotype 4: what we know and what we don't yet know. Hepatology 2008;47:1371–83.
[22] Rajaguru R, Nettleman MD. Hepatitis C infection, causes, transmission, symptoms, diagnosis and treatments: how does liver damage occur in hepatitis C infection? Egypt J Med Hum Genet 2011;96:1127–34.
[23] Alter HJ, Seeff LB. Recovery, persistence and sequelae in hepatitis C virus infection: a perspective on long-term outcome. Semin Liver Dis 2000;20:17–35.
[24] Thimme R, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, et al. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001;194:1395–406.
[25] Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci 2006;3:47–52.
[26] Ali S, Ali I, Azam S, Ahmad B. Frequency distribution of HCV genotypes among chronic hepatitis C patients of Khyber Pakhtunkhwa. Virol J 2011;8:193.

[27] Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011;17:107–15.
[28] Maheshwari A, Thuluvath PJ. Management of acute hepatitis C. Clin Liver Dis 2010;14:169–76.
[29] Ray Stuart C, Thomas David L. Hepatitis C. In: Mandell Gerald L, Bennett John E, Dolin Raphael, editors. Mandell, Douglas and bennett's principles and practice of infectious disease (7th ed.). Philadelphia, PA: Churchill Livingstone 2009;154(7).
[30] Jara P, Resti M, Hierro L, Giacchino R, Barbera C, Zancan L, et al. Chronic hepatitis C virus infection in childhood: clinical patterns and evolution in 224 white children. Clin Infect Dis 2003;36:275–80.
[31] Cantaloube JF, Laperche S, Gallian P, Bouchardeau F, de Lamballerie X, de Micco P. Analysis of the 5′ non coding region versus the NS5B region in genotyping hepatitis C virus isolates from blood donors in France. J Clin Microbiol 2006;44:2051–6.
[32] Wilkins T, Malcolm JK, Raina D, Schade RR. Hepatitis C: diagnosis and treatment. Am Fam Physician 2010;387(11).
[33] Ozaras R, Tahan V. Acute hepatitis C: prevention and treatment. Expert Rev Anti Infect Ther 2009;98(3).
[34] Farhana M, Hussain I, Haroon TS. Hepatitis C: the dermatologic profile. J Pak Assoc Derm 2009;18:171–81.
[35] Chevaliez S, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV related liver disease. Int J Med Sci 2006;3:35–40.
[36] Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36(Suppl. 11).
[37] Shier MK, Al-Wetidy MS, Ali HH, Al-Qattan MM. Characterization of hepatitis C virus genotypes by direct sequencing of HCV 5′UTR region of isolates from Saudi Arabia. PLoS ONE 2014;9:e103160.
[38] Idrees M. Development of an improved genotyping assay for the detection of hepatitis C virus genotype and subtypes in Pakistan. J Virol Methods 2008;150:50–6.
[39] Simmonds P. Genetic diversity and evolution of hepatitis C virus-15 year on. J Gen Virol 2004;85:3173–88.
[40] Zein NN. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev 2000;13:223–35.
[41] Simmonds P. The origin and evolution of hepatitis viruses in humans. J Gen Virol 2001;82:693–712.
[42] Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infect Dis 2008;8:69.
[43] Alter MJ. Prevention of spread of hepatitis C. Hepatology 2002;36:93–8.
[44] Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015;61:77–87.
[45] Chaudhary IA, Samiullah U, Khan SS, Masood R, Sardar MA, Mallhi AA. Seroprevalence of hepatitis B and C among the healthy blood donors at Fauji Foundation Hospital, Rawalpindi. Pak J Med Sci 2007;23:64–7.
[46] Zarkesh-Esfahani SH, Kardi MT, Edalati M. Hepatitis C virus genotype frequency in Isfahan province of Iran: a descriptive cross sectional study. Virol J 2010;7:69.
[47] Cenci M, Massi M, Alderisio M, Soccio GD, Recchia O. Prevalence of hepatitis C virus genotypes and increase of type 4 in central Italy: an update and report of a new method of HCV genotyping. Anticancer Res 2007;27:1219–22.
[48] Ali A, Nasir M, Ahmad H, Saif N, Idrees M, Bajwa MA. Determination of HCV genotypes and viral loads in chronic HCV infected patients of Hazara Pakistan. Virol J 2011;8:466.
[49] Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57:1333–42.
[50] Campiotto S, Pinho JRR, Carrilho FJ, Da Silva LC, Souto FJD, Spinelli V, et al. Geographic distribution of hepatitis C virus genotypes in Brazil. Braz J Med Biol Res 2005;38:41–9.
[51] Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med 2013;6(20).
[52] Sheet F. Hepatitis C. Geneva, Switzerland: World Health Organization; 2013. Available from <http://www.who.int/mediacentre/factsheets/fs164/en/>.
[53] Halliday J, Klenerman P, Barnes E. Vaccination for hepatitis C virus: closing in on an evasive target. Expert Rev Vac 2011;10:659–72.
[54] De Clercq E. Dancing with chemical formulae of antivirals: a panoramic view (part 2). Biochem Pharmacol 2013;13:67–96.
[55] Suzuki S, Izumi N. A triple combination therapy of simeprevir, pegylated-interferon and ribavirin with chronic hepatitis C virus genotype 1 infection. Nihon Rinsho 2015;73:266–72.
[56] Foster G, Mathurin P. Hepatitis C virus therapy to date. Antiviral Ther 2008;421:1109–21.
[57] Yu JW, Wang GQ, Sun LJ, Li XG, Li SC. Predictive value of rapid virological response and early virological response on sustained virological response in HCV patients treated with pegylated interferon alpha-2a and ribavirin. J Gastroenterol Hepatol 2007;22:832–6.
[58] Liu H, Liu CJ, Lin CL, Liang CC, Hsu SJ, Yang SS, et al. Pegylated interferon alpha-2a plus ribavirin for treatment naïve Asian patients with hepatitis C virus genotype 1 infection: a multicenter randomized controlled trial. Clin Infect Dis 2008;47:1260–9.
[59] Manns MP, Mchutchison JG, Gordon SC. Peg-interferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001;358:958–65.
[60] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, et al. Peg-interferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–82.
[61] Kmal SM, El-Tawil AA, Nakano T, He Q, Rasenack J, Hakim SA, et al. Peg-interferon alpha-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. Gut 2005;54:858–66.
[62] Hasan F, Askar H, Al-Khaldi J, Siddique I, Al-Ajmi M, Owaid S, et al. Peg-interferon alpha-2b plus ribavirin for the treatment of chronic hepatitis C genotype 4. Am J Gastroenterol 2004;99:1733–7.
[63] Fung J, Lai CL, Hung I, Young J, Cheng C, Wong D, et al. Chronic hepatitis C virus genotype 6 interferon: response to pegylated interferon and ribavirin. J Infect Dis 2008;198:808–12.
[64] Gilani SI, Ali S, Mir ST, Mazhar T, Iqbal A, Ahmed F, et al. Use of alternative medicine for chronic hepatitis C-A hospital based study from Rawalpindi. RMJ 2009;34:102–4.
[65] Zuo G, Chen L, Xu X. Activity of compounds from Chinese herbal medicine Rhodiolakirilowii (Regel) Maxim against HCV NS3 serine protease. Antiviral Res 2007;1:86–92.
[66] Friedman ND, Green JH, Weber HM, Stephen S, Lane SE, Ting AY, et al. Hepatitis C virus treatment in the 'Real-World': how well do 'Real' patients respond. J Clin Exp Hepatol 2014;4:214–20.
[67] Yu CI, Chiang BL. A new insight into hepatitis C vaccine development. J Biomed Biotechnol 2010:548280.
[68] Major ME. Prophylactic and therapeutic vaccination against hepatitis C virus: developments and future perspectives. Viruses 2009;1:144–65.
[69] Drane D, Maraskovsky E, Gibson, et al. Priming of CD4+ and CD8+ T cell responses using a HCV core ISCOMATRIX vaccine: a phase I study in healthy volunteers. Hum Vac 2009;5:151–7.
[70] Yotani S, Komatsu N, Shichijo S, Yoshida K, Takedatsu H, Itou M, et al. Phase I clinical study of a peptide vaccination for hepatitis C virus-infected patients with different human leukocyte antigen-class I-A alleles. Cancer Sci 2009;100:1935–42.

[71] Barnes E, Folgori A, Aston S. Phase I trial of a highly immunogenic T-cell vaccine for HCV based on novel adenoviral vectors from rare serotypes. Hepatology 2009;4(Suppl):105A.

[72] Sallberg M, Frelin L, Diepolder H, Jung MC, Mathiesen I, Fons M, et al. A first clinical trial of therapeutic vaccination using naked DNA delivered by in vivo electroporation shows antiviral effects in patients with chronic hepatitis C. Lancet 2009;31:23–6.

[73] Janssen HL, Brouwer JT, Van der Mast RC, et al. Suicide associated with alpha interferon therapy for chronic viral hepatitis. J Hepatol 1994;21:241–3.

[74] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558–67.