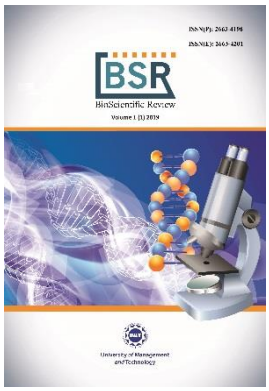

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Current Status of Therapeutics and Diagnosis of HCV: A Review

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Abstract

Hepatitis C virus (HCV) causes a very common blood borne infection. According to a recent estimate, 3% of world population is infected with HCV. Acute infection develops into chronic infection that causes severe liver diseases. Major improvements in diagnosis and antiviral therapy play a crucial role in the management of chronic hepatitis infection. Better understanding of HCV life cycle introduced the development of direct acting antiviral drugs (DAA drugs). Currently, sovaldi or NS5B inhibitor is a major drug used for chronic HCV infection. New therapies are based on the combination of antiviral drugs and/or interferon free regimens. Many new DAA drugs, that are inhibitors of HCV genes, are under investigation. Serological and molecular techniques play a major role in the diagnosis and assessment of the treatment. Anti HCV detection by ELISA is an initial screening test, while nucleic acid tests (NATs) are confirmatory. Quantitative NATs have replaced the qualitative NATs. Developments in the field of diagnosis and treatment have replaced interferon based regimens with interferon free regimens.

1. Introduction

Chronic Hepatitis C is a notoriously widespread blood borne infection of liver that has infected about 3% of the total world population. According to a recent survey, 185 million people worldwide are HCV infected and out of these 130 to 170 million people have developed chronic infection (1). More than 15 million individuals have been chronically infected with HCV in Asia and Africa, where its prevalence is high as compared to European countries (2, 3). With reference to the previously reported data, approximately 11 million individuals in Pakistan are HCV infected (4). In most of

the cases, infection remains asymptomatic. About 40% of patients recover completely, while about 70% acute infection develops into chronic infection which leads to further complications of liver, such as liver fibrosis, cirrhosis and hepatocellular carcinoma. Improved screening method can reduce HCV associated morbidity (5).

HCV virus belongs to family Flaviviridae and genus Hepacivirus. It has an envelope and positive single stranded RNA which codes large polyprotein, which after cleavage makes 10 different proteins and each protein performs its distinct function. Four structural proteins are core, p7, E1 and E2; while six other non-structural proteins include NS2, NS3, NS4A, NS4B, NS5A and NS5B that play a major role in viral replication. A better understanding of HCV life cycle led to the discovery of new therapies such as DAA drugs for chronic hepatitis that target different structural and non-structural proteins of virus proteins. The main function of DAA drugs is to inhibit viral replication. HCV has seven major genotypes and several subtypes (6).

Therapies of HCV infection are evolving rapidly and two drugs sofobuvir (sovaldi) and simeprevir (olysio) approved by FDA in 2013 have revolutionized the treatment. Present day treatment is based on the use of DAA drugs in combination with pegylated interferon and ribavirin. Previous therapies had many side effects that urged the scientists to develop the new one. The combination of sofosbuvir and simeprevir with ribavirin, is highly effective, especially against genotype 1 infection. Previous studies have shown sustained virological response (SVR) of more than 90%; Sovaldi has advantages over Olysio as it is effective for all genotypes and has a significantly low resistance (7). High cost of treatment, increased resistance, and the higher relapse rate associated with the use of interferon treatment raised the need of an alternative therapeutic approach and the development of latest DAA drugs is a major breakthrough that will help in the eradication of HCV (8, 9).

Baseline diagnosis involves both qualitative and quantitative tests which include first serological assay to detect anti-HCV by Enzyme Linked Immunosorbant Assay (ELISA) recombinant immunoblot assay (RIBA); both are very sensitive and specific assays. The second approach is the detection of virus RNA load by qualitative or quantitative PCR. Advanced molecular techniques involve nanoparticle technology, such as quantum dots or gold particles, to target specific HCV antigen. Liver biopsy is also recommended for the diagnosis of chronic HCV infection (10, 11).

2. Specific treatment for HCV patients

2.1. Pegylated interferon and ribavirin

Pegylated interferon in combination with ribavirin is the best treatment that is given to HCV patients. Traditionally, only a small proportion of patients have been cured by interferon alpha as a treatment. Thus, the combination with ribavirin has led to a re-appraisal of the management of chronic HCV. Pegylated interferon plus ribavirin dual therapy can clear the virus in 40-45% of previously not treated (naïve) patients, and in 5-21% of previously treatment failed patients. First generation protease inhibitor drugs are boceprevir and telaprevir, which significantly enhance the

probability of attaining a sustained virologic response to 63-75% and 59-66%, respectively in genotype 1. It is administered as triple therapy with the existing gold standard, that is, interferon. It has been reported that the combination therapy is the most beneficial for the treatment of HCV as compared to monotherapy (interferon alpha alone). Previously, a shortened peg-interferon and ribavirin combination therapy showed rapid virological response (RVR) in patients who had a low viral load (12).

Within the first few weeks of therapy, pegylated interferon provides a rapidly low level of HCV RNA, while ribavirin mostly affects ALT levels and acts like an immuno-modulator as opposed to a direct antiviral agent. Therefore, a weekly subcutaneous injection of pegylated interferon alpha along with ribavirin given twice daily has been the current standard care for chronic HCV. This combination has shown SVR in approximately 40–50 % of patients (13).

Interferon-naive patients and relapse patients are two groups of chronic HCV patients expected to benefit from the combination therapy. It has also been studied that the efficacy of pegylated interferon plus ribavirin (SVR=55%) was better than non-pegylated interferon plus ribavirin. Treatment with pegylated interferon either used as dual therapy or as monotherapy showed higher SVR rates than those treated with non-pegylated interferon. Patients with genotype 1 showed the lowest level of SVR as compared to patients with genotype 2 or 3 that showed the highest virological response. It seems that there is variation in response to therapy according to viral genotype. The combined SVR rates were 31% for pegylated interferon and 14% for non-pegylated interferon (14, 15).

There are several different adverse effects associated with the use of interferon based anti-viral treatment (such as flu like symptoms, vomiting, nausea and depression) and ribavirin (such as anemia). Patients have reported an unpleasant experience that disrupts their social and family life and impairs their ability to work. Two peg interferons (α--2a and α--2b) allow patients who have a low viral load (LVL) to achieve a rapid virological response (RVR) after 4 weeks of treatment (16).

2.2. Direct acting antivirals

Direct acting antivirals (DAAs) and host targeting antivirals (HTAs) are two advanced hepatitis C antiviral agents. They have revolutionized the field of HCV therapeutics by producing high SVR. They have reduced both the side effects and the time period of the treatment. On the other hand, HTAs are less effective although the first treatment for HCV was HTA, that is, interferon alpha. It also created a wide antiviral response but harmful side effects and resistance by the patients made it outdated (17).

The DAA drugs have increased the viral clearance rate up to 70%, as compared to 10% with interferon monotherapy. Therapy containing peg-interferon and ribavirin is not used anymore for the treatment of HCV genotype 1. HCV is treated with DAA drugs, a protease inhibitor combined with ribavirin and pegylated interferon; it is a mixture which has increased the likelihood of response to the treatment but has also showed a side effect of greater toxicity. Boceprevir and telaprevir were approved in 2011 as the

Genotype of HCV	Treatment Options Or Recommended Treatment	SRV (%)
1	Peg interferon + RBV	40 to 45 %
	Boceprevir +Peg-IFN + RBV	63% and 66 %
	Telaprevir +Peg-IFN +RBV	75% 81%
	Simeprevir+ Peg-IFN +RBV	89-93%
	Grazoprevir+ Peg-IFN +RBV	92% 97%
	Sofosbuvir+ Peg-IFN +RBV	98%
	SOF + SMV	
	SOF and VEL	
2	Peg interferon + RBV	70 to 75 %
	SOF + PEG + RBV	86 to 92 %
	SOF+ DVC	92 %
	SOF and VEL	98%
3	Peg interferon + RBV	70 to 85%
	SOF + PEG + RBV	85%
	SOF + DVC	89%
4	Peg interferon + RBV	50 t0 70 %
	SOF + PEG + RBV	96 %
	SOF + LDVSOF and VEL	100 %
	GZR and EBR	100 %
5 and 6	Peg interferon + RBV	60 to 90 %
	SOF + PEG + RBV	70 – 89 %
	SOF + LDV	100
	SOF and VEL	80%

Table 1. Treatment Response of Direct Acting Antiviral (DAA) Drugs in all HCV Genotypes Measured by Overall SVR

first DAA drugs and target the HCV, selectively. Until 2012, almost 30 DAA drugs were going through steps of clinical progression. **Table. 1** explains the SVR of different DAA drugs and the combination of these drugs against all 6 genotypes of HCV (5, 6).

2.3. NS3-4A Inhibitors

NS3-4A serine protease plays a key function in HCV life cycle as it cleaves the HCV polyprotein downstream region. It consists of two domains. One domain has serine protease activity, while the other has two major activities including RNA helicase and nucleotide triphosphatase activity. There are two major classes of NS3-4A inhibitors, non-covalent product based inhibitors and covalent reversible inhibitors.

Ciluprevir was the first NS3-4A inhibitor used in HCV patients. It was available for oral use. Ciluprevir growth was prohibited because of cardiotoxicity. Its research code was BILN 2061 (6, 9). Boceprevir and telaprevir are protease inhibitors used as a treatment for HCV genotype 1. It binds to HCV non-structural NS3 (HCV) active site. In 2011, telaprevir and boceprevir were accepted by US and Europe after being clinically developed. Triple therapy containing peg-IFN-α and ribavirin along with boceprevir and telaprevir are the most definite treatment used for HCV genotype 1 infection today. In case of triple therapy, the rate of viorological response is much higher as compared to peg-IFN-α and ribavirin. This triple therapy has increased the viral clearance rate upto 70%, as compared to 10% of interferon monotherapy. There are many more NS3-4A protease inhibitors which are in different stages of clinical trials such as danoprevir, faldaprevir, vaniprevir, asunaprevir, etc and in near future many more will be available. The benefits of these drugs over boceprevir and telaprevir

include better absorption, distribution and bioavailability. They have fewer side effects and require low dosing (7).

Limitations of triple therapy

- Restricted effectiveness for HCV genotypes 2-6
- Adverse effects
- Less effective in patients having an adverse liver disease
- Drug-drug interactions

2.4. NS5A RNA polymerase inhibitors

NS5A is a multifunctional zinc binding phosphoprotein that contains about 447 amino acids and accumulates the membranes which appeared from endoplasmic reticulum. It lacks any enzymatic activity but plays an important role in HCV replication. NS5A consists of three different structural domains (DI - DIII), including an amphipathic alpha helix at its N-terminus that is used in membrane localization (18). All the three domains of NS5A bind to RNA (19). Domain I has been crystallized as a homodimer. It contains 37-213 amino acids and is crucial for viral RNA replication. Domain II contains 250-342 amino acids and is linked with cyclophilin a binding site, thus easy to antagonize the innate immune response to HCV (20). Domain III contains 356- 447 amino acids and plays an important function in the assembly and packaging of infectious viral particles. Domain II and domain III are less well defined. Two types of NS5A known as p56 and p58 exist on the basis of electrophoretic mobility. The p56 form is basally phosphorylated in different regions by different kinases and p58 is hyper phosphorylated by casein kinase I isoform alpha (21). Because NS5A is engaged in multiple steps in HCV replication, NS5A inhibitors may have useful antiviral activity. NS5A inhibitors stop the replication of all HCV genotypes but their antiviral potency may differ against different genotypes, although it remains specific for the genotype 1 because of pan-genotypic reactivity (22). NS5A inhibitors have unknown specific structure of antiviral action. One assuming structure is the hyperphosphorylation inhibition. NS5A phosphorylation is essential for viral assembly (23) and hyperphosphorylation of NS5A seems to play distinct functions among *in vivo* and *in vitro* methods. The severity and need for it may vary during various extracts and genotypes (24). Various different DAA drugs are currently in clinical practice.

2.4.1. Daclatasvir (BMS- 790052)

Daclatasvir (BMS- 790052) is a very special inhibitor of the viral replication. This oral and once daily drug was developed by Bristol-Myers Squibb. It has indicated an effective reduction (3.6 log10) of HCV RNA and has no side effects in humans (25). It inhibits domain I and stops the NS5A hyper phosphorylation. It has the ability of changing the viral protein subcellular localization (26). It is highly effective against INF plus RBV combination and genotypes 1 and 2. It has determined 100% SVR post treatment in persons infected with genotype 4 at 12 weeks.

2.4.2. Ledipasvir (GS- 5885)

Ledipasvir (GS- 5885) is an oral and once daily drug established by Gilead. It is a strong NS5A inhibitor against genotypes 1a, 1b, 4a and 5a, but less specific against genotypes 2a and 3a (27).

2.4.3. ABT- 267

ABT- 267 is an oral and once daily NS5A inhibitor developed by AbbVie, which

lowers the HCV RNA levels *in vitro* and *in vivo*. However, NS protein is an essential switch of sudden viral targets such as viral RNA replication and binding, virus production and also conducts antiviral interferon response. All of these functions provide a different way for NS5A inhibitors to react with other common target based drug discovery. So, NS5A designed drugs are extremely important anti- HCV compounds ever recognized (28, 29).

2.5. NS5B RNA polymerase inhibitors

NS5B RNA dependent RNA polymerase is a viral replicating enzyme with a high error rate due to the lack of proofreading domain and exonuclease activity and this is why HCV has different genotypes. NS5B has a key role in viral replication as it uses positive strand RNA as a template and replicates the RNA. An active site of NS5B consists of aspartic acid residues, any molecule that binds with its active site and which can alter the activity of enzyme to inhibit viral replication. So, NS5B inhibitors are good therapeutic agents due to the inhibition of viral replication (5, 6). Two categories of NS5B inhibitors are known. These include nucleoside analog inhibitors (NIs) and non–nucleoside analog inhibitors (NNIs). NIs are better than NNIs in action and effective against all genotypes, while NNIs have a low resistance barrier and they do not bind to conserved region. Instead, they bind with enzyme allosteric sites and change the conformation of proteins involved in the formation of elongation complex. Contrarily, NIs are incorporated as a substrate in RNA chain and terminate the chain.

Valopicitabine was the first NI but it had low antiviral activity and high toxicity. RI626 was another analog which shows good antiviral action but also causes severe lymphocytopenia. Others NIs are RG7128, IDX184 etc. A major NS5B polymerase inhibitor sovaldi (PSI-7977), also known as sofosbuvir, is an oral DAA drug and a pyrimidine analog approved by FDA in 2013. Its administration dose is 400mg/day. It lessens the viral load rapidly, has excellent safety, a high barrier to resistance and should not be combined with rifabutin and carbazpine; although its combination with ladipasvir gives very promising results (7, 8).

In December 2014, FDA approved Viekira Pak that was very effective against genotype 1 and cirrhosis patients. Viekira Pak tablet consists of ombitasvir, paritaprevir, ritonavir tablets and dasabuvir. Dasabuvir is another polymerase inhibitor which is non-nucleoside and is used as an interferon free combination therapy. Beclabuvir, ABT-07, Mericitabine, VX-135, L-335, AL-516, GS-6620,ABT-333, Lomibuvir, Filibuvir (PF-868554), GSK2878175, Tegobuvir and Deleobuvir all are polymerase inhibitors that are currently in use (9).

2.6. Epclusa (Sofosbuvir and Velpatasvir)

This is an interferon free medication for all 1- 6 genotypes and mixed genotypes of HCV. Recent advances have shown that epclusa is a DAA drug which is made by the combination of sofosbuvir (NS5B polymerase inhibitor) and velpatasvir (NS5A inhibitor). It is taken as a tablet per day with or without ribavirin prescription. It is administered as a short course, which lasts for only twelve weeks. Patients who are already suffering from Bradycardia and Hepatitis B virus should not take it. Its

SVR is 93% – 99%. It adversely affects the patients (headache and fatigue) who are taking it without ribavirin. However, in conjugation with ribavirin, it causes severe anemia, nausea, diarrhea, insomnia and tiredness (30 - 33)

3. Base line tests for diagnosis

Basically, there are two main types of diagnostic tests recommended for the management of HCV, that is, serological assays involving HCV antibody analysis and nucleic acid tests (NATs) involving the viral RNA load analysis. These assays can be qualitative and quantitative tests. Moreover, there are three generations of all these diagnostics tests according to their level of sensitivity and specificity (34-38).

3.1. Serological assays

Screening the presence of virus serological assays involves enzyme linked immune sorbent assay (ELISA) or recombinant immunoblot assay (RIBA) (34). The first generation serological tests used the non-structural NS4 gene product of virus as an antigen involved in pathology.

The second generation assays were established with more sensitivity by involving the use of core and NS3 gene products in addition to NS4. This second generation was then replaced by a more sensitive third generation serological assays using core, NS3 and NS5 gene products as antigens. This third generation enzyme immunoasssays (EIAs) have a sensitivity of about 98%. Latest fourth generation is also available that has a better sensitivity and detects the capsid antigen and antibodies to core and NS3, NS4, NS5 genes of the virus. HCV core antigen detection is an attractive diagnostic tool that requires less time. Since the day of their discovery, they have improved a lot in performance (35, 36).

3.2. Molecular assays

The second type of tests that play a crucial role in the diagnosis and monitoring of treatment are molecular assays. They are also called NATs (Nuclear Acid Tests). These tests can detect and quantify viral RNA even after the first week of virus exposure. It is a gold standard test for the detection of active HCV replication as virus cannot be cultivated (37). These diagnostic tests are classified as qualitative and quantitative. Qualitative tests are confirmatory tests for HCV diagnosis that include qualitative PCR and TMA (Transcription Mediated Amplification). Quantitative assays measure the quantity of virus in a patient's blood. The results of quantitative assays give valuable information about the initial viral load, reduction in virus with therapy and detection of SVR. RNA level in blood is not associated with liver damage, infection period and extremity of disease (38). Quantitative tests include bDNA (branched Deoxyribonucleic Acid), RT-PCR, real time PCR, and hybridization and chemilluminescent assays (39). These assays also have three generations on the basis of their better sensitivity (99%) and specificity (about 99%). Due to the above stated attributes, quantitative tests have replaced the qualitative tests (40- 41).

3.3. Genotype testing

Genotyping is a well-known primary tool to determine the infection and duration of treatment and the response of patient to the specific treatment. The HCV genotype detection has remained an ongoing area of research lately but there have developed many techniques to provide an accurate genotype detection. These include 5′-

restriction fragment length polymorphism, core gene nested PCR, El gene or NS5B gene phylogenetic studies and sequencing and reverse hybridization or line probe assay (LIPA) assay (42- 45).

4. Recent advancement

In recent years, there has been considerable progress in the establishment of advanced molecular techniques, which has enabled the detection of HCV genotype more accurately. These advanced molecular assays involve nanoparticle technology, using quantum dots and gold nanoparticles to target HCV antigens. Other new diagnostic approaches include aptamer diagnostics, some point of care methods, that is, loop-mediated isothermal amplification (LAMP) and polydimethylsiloxane (PDMS) micro-devices (46-52).

5. Future perspectives

Many new DAA drugs are in the clinical phase with the hope that in coming years interferon free regimens will dominate HCV treatment. There are about 30 to 40 new drugs under investigation for HCV treatment. The major goal is to reduce the morbidity and mortality rate by achieving high SVR and the selection of drugs with minimized resistance, less side effects and high efficiency. Many protease and polymerase inhibitors show better efficacy, less resistance and more safety in clinical investigation. Another major factor to be controlled in the future is the high cost of available DAA drugs that is still a barrier for poor people and it can be eradicated with the development of pangenotypic drugs. Epclusa is a pangenotypic drug that has been approved by FDA but it is still not available in developing and under developed countries (31, 32).

6. Conclusion

The major factor responsible for hepatocellular carcinoma is HCV. The vast majority of HCV infections lead to chronic liver diseases. Interferon in combination with ribavirin was the standard of care therapy for either 24 or 48 weeks than interferon alone in inducing virologic and histologic improvement. Thus, the combination of pegylated interferon and ribavirin may therefore be indicated as an initial therapy in HCV patients. With this regimen, the rates of SVR were approximately 15 to 20%. SVR is significantly increased in patients infected with HCV genotype 1 by the addition of boceprevir in combination with peginterferon alfa-2b–ribavirin therapy. DAA drugs have revolutionized the area of HCV therapeutics by producing high virological response. The efficacy of therapy depends on numerous viral and host factors and can be limited by adverse events. Nucloside, nucleotide and non-nucleoside are the three subclasses of polymerase inhibitors; the lowest antiviral activity and the highest risk of resistance is shown by non-nucleoside polymerase inhibitors. When they combine with more potent anti-HCV drugs, they can serve as important additional compounds. Diagnostic tests include serological assays involving HCV antibody analysis and nucleic acid tests (NATs) involving the viral RNA load analysis. Serological assays such as ELISA and RIBA are performed in which antigen-antibody binding is carried out. Molecular assays are used for diagnosis, monitoring and detection of active HCV replication because virus cannot be cultivated. These assays have three generations on the basis of their better sensitivity and specificity and due to these attributes they have replaced the qualitative tests. In brief,

efforts to develop therapies that are more effective must remain a high priority despite recent progress. The best hope for a solution to the epidemic of HCV infection worldwide is the development of an effective vaccine. A new therapeutic approach can be expected in the future for those who are already infected with HCV.

Competing interest

None

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None

7. References

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