Evidence-based consensus on the diagnosis, prevention and management of hepatitis C virus disease

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Abstract

Hepatitis C virus (HCV) is a potent human pathogen and is one of the main causes of chronic hepatitis round the world. The present review describes the evidence-based consensus on the diagnosis, prevention and management of HCV disease. Various techniques, for the detection of anti-HCV immunoglobulin G immunoassays, detection of HCV RNA by identifying virus-specific molecules nucleic acid testings, recognition of core antigen for diagnosis of HCV, quantitative antigen assay, have been used to detect HCV RNA and core antigen. Advanced technologies such as nanoparticle-based diagnostic assays, loop-mediated isothermal amplification and aptamers and Ortho trak-C assay have also come to the front that provides best detection results with greater ease and specificity for detection of HCV. It is of immense importance to prevent this infection especially among the sexual partners, injecting drug users, mother-to-infant transmission of HCV, household contact, healthcare workers and people who get tattoos and piercing on their skin. Management of this infection is intended to eradicate it out of the body of patients. Management includes examining the treatment (efficacy and protection), assessment of hepatic condition before commencing therapy, controlling the parameters upon which dual and triple therapies work, monitoring the body after treatment and adjusting the co-factors. Examining the treatment in some special groups of people (HIV/HCV co-infected, hemodialysis patients, renal transplanted patients).

Key words: Hepatitis C virus; Enzyme immunoassay; Nucleic acid testing; Loop-mediated isothermal amplification; Sustained viral response; Telaprevir; Boceprevir; Liver transplant

Core tip: The present review describes the evidence-based consensus on the diagnosis, prevention and management of hepatitis C virus (HCV) disease. Besides the conventional techniques more advanced technologies have come to the front that provides best detection with greater ease and specificity. It is of immense importance to prevent this infection among the sexual partners, injecting drug users, mother-to-infant transmission of HCV, household contact, healthcare workers and people who get tattoos and piercing on their skin. Management includes examining the treatment, assessment of hepatic condition before

commencing therapy, controlling the parameters upon which dual and triple therapies work, monitoring after treatment and adjusting the co-factors.

INTRODUCTION

Hepatitis is a viral infection of the liver that ultimately causes liver to become swollen thus inflammation occurs. Hepatitis C is caused mostly due to viral agent, smoke, pollution and unhygienic condition of the surrounding[1]. Hepatitis C virus (HCV) is an infectious particle that causes cirrhosis and carcinoma of hepato-cellular components round the globe[2]. HCV prevalence is 4.95% in general and 57% in injecting drug use (IDU) population of Pakistan[3]. HCV is small in size measuring 55-65 nm. It is a positive stranded RNA virus and belongs to the family of *Flaviviridae*. Its RNA genome constitutes a single open reading frame made up of 9600 nucleotide bases long. In addition to envelope proteins E1 and E2, core protein comprises structural proteins and non-structural proteins include NS1, NS2, NS3, NS4a, NS4b, NS5a and NS5b[4].

Mainly, six genotypes exist for HCV isolates having a difference of 30%-35% in their nucleotide sequences and multiple subtypes differ up to 20% to 25%[5]. 1a and 1b genotypes are the most prevalent ones in the Western Europe and United States then 2 and 3 genotypes come next in the order. While genotype 4 is widespread in Egypt, genotype 5 is common in South Africa, and genotype 6 is in Southeast Asia[6]. In patients from Canada and Belgium, another seventh genotype has also been identified[7].

Three common assay procedures have been used to diagnose the infection. These comprise some anti-HCV antibody assay, detection of HCV-RNA and very recently Hepatitis C Virus core antigen assay[8].

This review will focus on the diagnosis, prevention and management of HCV infection.

DIAGNOSIS

Enzyme immunoassay (EIA) and HCV RNA assay are performed to detect the presence of anti-HCV antibodies in suspected patients of acute hepatitis C. HCV RNA assay is a sensitive technique with a lower detection limit of 50 IU/mL or it can be less than this value[9]. In the absence of anti-HCV antibodies, the presence of HCV RNA is a pinpoint of acute HCV infection. It is further confirmed after a few days or weeks through seroconversion (*i.e.,* the emergence of

anti-HCV antibodies). At the time of diagnosis, both of HCV RNA and anti-HCV antibodies can be found in patients with acute hepatitis. In this case, it is not easy to distinguish acute hepatitis C with chronic hepatitis C exacerbating acute infection. If both anti-HCV antibodies and HCV RNA are absent then acute infection is improbable. When HCV RNA is absent but anti-HCV antibodies are present acute infection of HCV is unlikely. As HCV RNA can be undetectable temporarily so retest should be taken of these patients after a few weeks. It is mainly because immune response partially takes control of replication of virus before chronic hepatitis C infection occurs[10]. This case is observed also in the patients who have recovered from long-ago HCV infection.

In case of chronic hepatitis C, both HCV RNA and anti-HCV antibodies (detecting 50 IU/mL or less with a sensitive technique) must be present[11,12]. Replication of HCV is detectable only by the third-generation EIAs that has been profoundly observed in hemodialysis and immunodepressed patients[13,14]. In a research study, B-cell epitopes and antigenic regions were recognized after cloning the genome of the HCV[6]. The development of screening tests for anti-HCV IgG, requires synthetic peptides including the epitopes that were immunodominant and recombinant proteins[15,16]. Recently, Food and Drug Administration approved an anti-HCV IgG assay for clinical use in United States[17]. However, these tests are unable to identify active HCV infection in IgG positive patient. As this antibody may be detected in patients who have evaded viral infection. In order to diagnose active HCV infections, nucleic acid testing (NAT) can be carried out for the detection of HCV RNA. But it cannot be used in laboratories frequently as it requires specialized technical staff and expensive tools. However, it can be used to detect HCV RNA. For that matter, serological assays independent of NAT are mostly used for easy identification of HCV infection[18].

Detection of IgG against HCV

Several immunoassays have been developed for the detection of anti-HCV IgG in plasma or serum samples. A recombinant protein expressed in yeast containing an epitope from NS4 section of HCV genome was used to carry out first-generation assays. These were successful in identifying anti-HCV IgG in posttransfusion HCV patients (80%) and caused a significant decrease in the infections. But due to their lack in specificity and sensitivity[19], there was a need to establish second- and third-generation assays. These assays involved use of multiantigens and antigens of core, NS3 and NS4. These modifications proved good for enhanced sensitivity and specificity[16].

Recognition of specific molecules of virus through NATS

The detection of HCV RNA is the most reliable marker

for diagnosis HCV infection. HCV RNA is detectable within 1 wk after its exposure in serum or plasma. As described earlier, NATs recently in use for detecting its RNA work on the principle of polymerase chain reaction (PCR), transcription mediated amplification, and branched DNA signal amplification. Another set of assays named as Qualitative and quantitative PCR assays have been accepted for use in laboratory by regular authorities in Europe and United States for diagnosing HCV RNA. The qualitative assay is not used now but ultrasensitive quantitative NATs have a broad range and can even detect RNA as little as 5 IU/mL. Moreover, HCV genotyping is also applicable in this regard. It is done through restriction fragment length polymorphism analysis, direct sequencing and reverse hybridization to genotype-specific oligonucleotide probes[20].

Role of core protein in HCV diagnosis
This is another very prominent technique for the diagnosis of HCV infection. Core protein is an RNA-binding protein that makes the viral nucleocapsid. A host signal peptidase cleaves it from polyprotein at the C-terminus, generating an immature form of the core protein[21], and further processing of signal peptide present at the C-terminus of the core is done by a host signal peptide peptidase, yielding its mature form[22]. It is highly antigenic in its nature as it can evoke immune responses. HCV nucleocapsid (core) may participate valuably in the vaccine development as it is the most conserved viral antigen. Various studies showed the presence of core protein in nearly 80%-92% of HCV positive patients with anti-HCV antibody[8]. Therefore it can be used as a diagnostic marker. In few studies, HCV core assay is less sensitive than anti-HCV antibody assay or HCV-RNA assay. Quantification of total core antigen of HCV is a precise indirect marker of its replication in the infected people. The sensitivity of HCV-RNA test was found to be 99% that of HCV core assay was noted as 98%[23]. In fact, more studies are required to further validate the beneficial aspect of HCV core estimation over HCV-RNA detection for the identification of the infection. The role of the core protein can be determined more extensively relating to HCV entry, replication and virion production. The culture system may be proved to help in developing antiviral agents targeting different stages of virus cycle, and these may be potential therapeutic agents.

Quantitative antigen assay
In Europe, the Architect HCV Ag assay (Abbott) is commercially available. It employs an automated platform and is an immunoassay based on a chemiluminescence in which monoclonal antibody specific to the HCV core antigen are coated on microparticles[24]. It has been found from many studies that HCV core antigen can be detected within the first 2 wk of acute infection. It has shown to be 96% to 100%

specific and 80% to 99% sensitive. This assay has an advantage that it is an immunoassay and works like molecular assays in that it does not require processing of samples and active infection is confirmed through positive result. It has one disadvantage of being lower sensitive than NAT; approximately 1000 IU/mL of HCV RNA is its lower limit of detection. This assay is not available in United States yet.

Up-coming technologies for HCV detection
A research study showed that biomarkers can be detected by the recently developed prototype nanoparticle-based diagnostic assays in diseases like HCV. Gold nanoparticles and quantum dots (QDs) are the most commonly used nanoparticles[25]. QDs are composed of semiconductor substances that upon excitation give off light at different spectra[26]. Using biochip-based assay 1 ng/mL was obtained. An oligonucleotide probe containing RNA conjugated with quantum dots targeting NS5B protein of hepatitis C virus[27]. Moreover, gold nanoparticles varying from 2 to 50 nmol/L in size have been used to detect HCV RNA and anti-HCV[28]. Antibodies to HIV, HBV, HCV, have been detected through a multiplex platform that uses a micro-fluidic chip and quantum dots coated with antigen are embedded in beads made of polystyrene having a sensitivity of pM concentration[29,30]. Another important method working on the principle of amplification, named loop-mediated isothermal amplification (LAMP) has been used for detection of HCV RNA[31,32]. Several other techniques have been estimated for the diagnosis of HCV. Currently used technology is aptamers which are used as capture molecules. These are small, single-stranded oligonucleotides that can be folded into three-dimensional structures and able to identify target molecules including proteins, cells, and chemicals[33]. They have the potential to bind with their targets with high specificity and attraction[34].

Ortho trak-C assay
According to a research conducted by Fabrizi *et al*[35], an immunoassay that quantitatively measures the total HCV core Ag irrespective of the presence or absence of anti-HCV antibodies in human serum or plasma was used. It is a manual method that uses several monoclonal antibodies specific to various regions of the core antigen of HCV. It employed a microwell plate and onto which monoclonal antibodies were coated that bound with antigen. Horseradish peroxidase was conjugated with Fab segments of monoclonal antibodies and bound with captured antigen. The method comprised four steps: In the first step, incubation of the control material or patient sample (100 μL) in an uncoated microwell was done with a pretreatment reagent (50 μL) at 56 ℃ for 30 min. So as to facilitate the liberation of the core antigen of HCV to be detected, this pretreatment step was undergone

for the dissociation of any immune complexes. In the second step, the dilution and incubation of pretreated sample (100 μL) was done at 25 ℃ for 60 min in a monoclonal antibodies coated microwell that captured the immunoreactive core antigen. In order to remove any unbound material the wells were washed. In the third step, the conjugate (200 μL), containing Fab fragments of monoclonal antibody conjugated to hor-seradish peroxidase, was added into the microwell. The mixture was incubated at 25 ℃ for 30 min. The conjugate got bound with the core antigen that was bound to the capture antibody coating the surface of microwell. The core antigen connected the capture and conjugate antibody reagents. Again washing was done at the end of the step to remove any unbound antigen. The fourth step was based on an enzyme detection system consisted of hydrogen peroxide and *o*-phenylenediamine (OPD) that was added to the microwell. The *o*-phenylenediamine was oxidized, in the presence of bound conjugate that produced a colored end product. In this reaction, hydrogen peroxide divalently oxidized the peroxidase producing an intermediate compound that was reduced to initial state after interacting with hydrogen-ion-donating OPD. As a result, orange coloration of oxidized form of OPD was produced. In order to stop the reaction, sulfuric acid was added. The intensity of the color is proportional to the amount of bound conjugate and thus it can be said that it is a function of the amount of core antigen of HCV in the sample.

Through a microwell reader (photometer) designed to measure absorbance of light in a microwell (at 490 nm, using a 620 nm reference), the intensity of the color is measured. The samples and controls were tested in duplicate, and the mean optical density (OD) of the duplicate tests was used. Variation more than 25% between the optical densities of two samples pointed towards invalidity of those samples and they were allowed to be re-tested. If optical density more than recommended cut off corresponding sample of was obtained only then the sample was considered positive. The concentration of core antigen of HCV was measured through a standard curve[35].

PREVENTION OF HCV

Approximately 3% of the population of world is affected by HCV and according to estimation last stage of liver disease develops in 30% of patients[36]. Several research studies are being conducted for developing new therapeutic and prophylactic vaccines for hepatitis C virus. However, these efforts to produce effective vaccines have been damaged due to some of characteristics in common with many RNA viruses and the reasons are: First is RNA viruses display high antigenic and genetic diversity thus the mutation rate is very high inside the host. Second is the induction of strong immune responses that cannot eradicate

the virus or avoid re-infection. Third is that there is no cell culture system developed so far or small animal model to develop vaccines. Thus so far, the vaccines developed have been tested on chimpanzees but they are unable to avoid HCV infection in them[37].

As there is no vaccine available for HCV so its prevention is even more difficult than HBV. It needs to have an integrated strategy including safe injection practices, and screening of blood donations[38]. The incidence of HCV infection is at its elevated level among the people who inject drugs (PWID)[39]. Nelson *et al*[41] estimated that in 2010, almost 10 million PWID were positive with HCV antibody and the global prevalence of HCV was 67% in that population[40]. Martin et al has used mathematical modeling to obtain the consequence of collaborative high-coverage needle and syringe programs (NSPs), opiate substitution treatment (OST) among PWID and treatment of HCV on its incidence and prevalence. For the preven-tion of HCV Page et al has emphasized upon the challenges of behavioral interference[39]. Many of the researchers have discerned about less effectiveness of harm-reduction programs in the prevention of human immunodeficiency virus (HIV) and HCV infection among the people who inject drugs. Many other strategies are still required. Results from their work have shown that from the last 10 years the prevalence of chronic HCV can be reduced up to more than 45% further needs antiviral treatment combined with the NSPs and OSTs that can reduce the rate of treatment to a large extent to get reduction in prevalence of HCV. In order to prevent HCV infection, its vaccine should become available. Two researchers named Cox and Thomas have stressed upon the development of HCV especially for people who inject drugs. Advanced research would be required for vaccine production[42].

Following are the modes of transmission of hepatitis C which cause people at risk of acquiring this infection:

Transmission of HCV infection through sexual contact
People at threat: Sexual transmission of HCV does not appear to be so common, as the studies has proved its spread in < 1% of couples yearly, among monogamous heterosexual partners. Meanwhile, researchers have currently discovered several cases of male sex partners (MSM) infected acutely with HCV and found it to be among men. Majorly these male sex partners had also HIV co-infection. However, the risk factor that is considered to be the strongest in the acquisition of HCV is injecting drug use. Many of the cohort and case studies have identified the prevalence and incidence of HCV among HIV-negative MSM, non-injecting drug users to be low apparently.

Factors related to elevated threat of sexually transmitted HCV: Acquisition of HCV has an enhanced risk associated with it in case of heterosexuals that have multiple sexual partners. Several risk factors have

Table 1 Approaches to prevent intra-venous drug users infection among hepatitis C virus

Reduce the risk of viral transmission among IDUs
Doctors should be educated properly
Lessen the use of injecting drug use
Use of sterilized syringes and other instrument even for diagnosis
Medical staff should be informed to inject safely
Hepatic disorder should be decreased in infected IDUs
Proper counseling should be undergone
Antiviral therapy for HCV
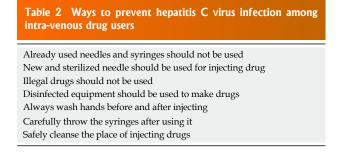Combination of all kinds of services should be provided to infected patients

IDUs: Intra-venous drug users; HCV: Hepatitis C virus.

Table 2 Ways to prevent hepatitis C virus infection among intra-venous drug users

Already used needles and syringes should not be used
New and sterilized needle should be used for injecting drug
Illegal drugs should not be used
Disinfected equipment should be used to make drugs
Always wash hands before and after injecting
Carefully throw the syringes after using it
Safely cleanse the place of injecting drugs

been identified by investigators that are related to HCV transmitted sexually among male sex partners: (1) HIV co-infection; (2) during sex, use of leisure drugs, *e.g.,* methamphetamine and gamma-hydroxybutyric acid (GHB); (3) unprotected intercourse through anus; (4) the sexual attempts that cause damage or bleeding from genital mucosa, group sex and fisting; and (5) ulcerative diseases that are transmitted sexually (syphilis, lymphogranuloma venereum proctitis) co-incidently[43-46].

Transmission of HCV via injection drug use
Infection of HCV among IDUs: In several parts of the world, injection drug use has become the most widespread threat factor for current cases especially in the United States. Acquisition of HCV is quite rapid when IDU is started meanwhile within short time interval a person becomes easy prone towards HCV infection. The occurrence of antibody of hepatitis C virus HCV antibody in IDUs enhances with many factors including age, intensity of injection, frequency of use and time from when injecting was initiated. As per a potential survey done form 2000-2007, HCV infection has an incidence of 27 out of 100 persons annually[47]. Re-infection has been observed in 26% patients who had previously eliminated the initial infection. Many co-researchers have demonstrated re-infection and super-infection[48]. Table 1 shows different approaches to prevent HCV infection among IDUs.

Factors Related to Transmission of HCV *via* Injection Drug Use: Injection Drug Use is considered to be a risk factor for the transmission of HCV. It spreads through sharing of syringes and needles. Moreover, many researchers have described that the equipment (*e.g.,* drug cookers, rinse water and filtration cottons) shared with the infected person is used in the making and injecting drugs. There is a dire need for counseling so to evade the use of contaminated syringes, needles and already prepared drug along with equipment. As an interesting fact, the persistency of HCV in syringes that are contaminated and having a large residual volume is very high[49]. According to a research study the survival of HCV is 16 h at room temperature outside the body but in the environment it cannot survive after 4 d[50]. Table 2 indicates different ways to prevent HCV infection among IDUs.

Transmission of HCV from mother-to-child
Risk of mother-to-child transmission: Transmission of infection from mother-to-child is more common in cases of hepatitis B or HIV than that in hepatitis C, but it occurs in its case also. This type of transference is apparent mostly in HCV viremic women. These women have detectable amount of RNA in the peripheral blood. According to research, the transmission of HCV through mother-to-infant was found to be 4.3% from year 1992 to 2000 and a study conducted by Pembrey *et al*[51] depicted the rates of transmission from 3%-10%.

Factors associated with HCV transmission from mother-to-child: The most common risk factor that becomes the cause of HCV transference from mother-to-child is co-infection of HIV in mother with HCV viremia detectable during pregnancy[52-54]. Several other factors have also been observed and they are; injection drug use by mother, if infant is a female, intrapartum events leading to exposure of infant maternal blood infected with HCV, rupture of membrane for a long time while managing labor and obstetric procedures like vaginal/perineal lacerations. Contrary to it this transmission has not been found to be related to breast feeding or type of delivery whether Cesarean or vaginal[55]. If breast feeding is being done safely (if there is no damage or bleeding to the nipples) then it can reduce the risk of HCV transmission or breast feeding can be halted if there is any crackled nipple) until it gets healed.

Transmission of HCV through household
Risk of transmission: Some studies related to epidemiology have proved that HCV infection can also be caused by having family contacts of seropositive persons. Exposure to shared parenteral things is a critical factor that includes vertical transmission from mother to infant; dental or medical processes or injections; or if spouses and partners have any sexual exposure. HCV infection was found to increase among siblings and family members of patients that were infected with chronic liver disease. In case if children were infected with HCV there was no elevated threat

of this infection was found in parents or siblings. In several research studies the increased risk among partners and family members had a correspondence with the intensity of liver disease in the infected patient, any sexual contact with him, the period of exposure to him and the number of contacts infected with hepatitis c virus[56].

Transmission in healthcare units
Hepatitis C virus has been a serious problem in centers where hemodialysis is done[57,58]. Many steps have been taken by these centers in order to prevent hepatitis C virus infection, which are: HCV infected patients are grouped or isolated in separate rooms of dialysis center, adherence to infection-control rules has been increased for example the screening for HCV at regular intervals, not to reuse the shared vials or syringes and regularly vaccinating HCV infected patients with hepatitis A and B virus[57]. Resultantly, HCV infection has been reduced in dialysis centers. But the problem is that every healthcare setting has not taken these steps. Another factor which is challenging for most developing countries is that there is no viral hepatitis control program at the Ministry of Health level. Due to the absence, indirect and distorted trials for prevention are undergone for all types of hepatitis not just for HCV. Therefore, to counter transmission of HCV especially in healthcare units, infection control programs are required.

Transmission through tattooing and piercing
In United States, there is no outburst of HCV infection has been notified by CDC as per licensed tattooing or piercing commercially. Urbanus *et al*[59] observed it for several years and pointed out that these practices seemed to be safe[59,60]. However, like everywhere else, exceptions are also here. Miller *et al*[61] and Sameul *et al*[62] separately found that in United States and Australia, the prisoners who got handmade tattooing were infected with HCV. Tohme *et al*[60], thus, declared that noncommercial tattoos if received in places like homes, unsterile environment or friends might become the cause of transmitting this infection.

MANAGEMENT OF HCV INFECTION

Aim of treatment of hepatitis C virus
It is the aim of this therapy to wipe out HCV infection so to avoid various liver and extra-hepatic disorders *e.g.,* cirrhosis, fibrosis, hepato-cellular carcinoma, inflammation due to necrosis and mortality.

Endpoint of HCV treatment
Sustained virological response (SVR) is the endpoint of HCV treatment; it is characterized by 24th week non-detection of HCV RNA. It was assessed by a molecular method that had detection limit of < 15 IU/mL (SVR24). Approximately 99% of cases have been cured as

explained by long-term follow up SVR studies[63].

Pre-therapeutic estimation
In order to establish a connection between liver disease and HCV infection, baseline virological characteristics should be determined so to assess the severity of liver disease. This assessment is suggested before commencing the treatment. It is important that cirrhosis must be identified in patients as the treatment and post-treatment response is related to the fibrosis phase. As proposing the type and duration of therapy is based on the absence of major fibrosis. If cirrhosis has been clinically evidenced then there is no need to do biopsy for assessment of fibrosis. If patients suffer from cirrhosis then they need to be screened for hepato-cellular carcinoma. The two tests can be applied to identify cirrhosis and they are; Liver stiffness measurement (LSM) and biomarkers. Both LSM and biomarkers can be used to measure the fibrosis of liver in patients suffering with chronic infection of HCV. This combination therapy can decrease the requirement to do biopsy of liver[64,65].

Quantification of HCV
Quantification of HCV is recommended to the patients who are willing to receive anti-viral therapy. The quantification is made with the help of a sensitive technique, and the concentration should be given in IU/mL. Genotyping should also be done before starting the treatment. The rate of response to treatment depends on specific genotypes and then subtypes[66]. Every genotype and subtype shows different response to therapy. Then genetics of host should be determined as IL28B genotyping helps in identifying infected persons who will achieve rapid virological response (RVR) and possess a considerable probability to get cured by dual therapy[67,68]. For any treatment to be applied, patient's preferences must be considered primarily. In case when patients infected with HCV genotype 1 become unable to remove virus when treated with dual therapy then should be treated with triple therapy. For other genotypes, Interferon-alpha based therapy should be used along with dual therapy.

Contra-indication to interferon-alpha and ribavirin therapy
Hezode *et al*[69] found that pegylated interferon-α/Ribavirin containing schedule of treating chronic HCV infection showed contra-indication in the following infected groups of patients; people suffering from epilepsy or psychosis, depression, couples who do not comply with the rules of contraception or pregnant women, concomitant severe diseases; disastrous liver disease.

Triple therapy based on telaprevir or boceprevir
Usually, both above mentioned dual therapy containing pegylated interferon-α/Ribavirin and triple

therapy based on TVR or BOC offer common contra-indications. In case of compensated cirrhosis, intense care is required for its treatment due to emergence of dangerous side effects after getting treated. As incidence of severe infections and hematological disorders is enhanced largely in case of triple versus dual therapy, particularly if serum albumin level is less than 3.5 g/dL or concentration of platelets becomes less than 100000 even sooner than commencing treatment[69].

Examining treatment

To monitor the treatment requires checking the efficiency, safety and side effects of treatment.

Efficiency of treatment

The efficiency of treatment can be monitored by repeating quantification of levels of HCV RNA. Monitoring of treatment efficacy is based on repeated measurements of HCV RNA levels. A precise and sensitive test should be used in order to get dynamic range in quantification. To ascertain the reliability of results, RNA can be measured in each patient using the same assay at various time points[70-72]. But to examine the efficiency of treatment and directions to follow for the duration of treatment, measurements of RNA levels should be taken at particular time points. If in case the result of quantification has some effect on the treatment only then measurements should be made. The treatment should be curtailed as response-guided therapy if it is to be discarded as it has uselessness and if it becomes successful then it is end of treatment and post- treatment SVR test. In order to measure SVR, Baseline assessment of HCV RNA levels should be done in dual therapy (pegylated interferon-α/Ribavirin), *i.e.,* 4th, 12th, 24th week, at the end of treatment or 12/24 wk after the completion of therapy. While in case of triple therapy with boceprevir, RNA should be quantified with the same schedule as that of dual therapy. It is referred in many Guidelines that the quantification of RNA with BOC therapy should be done after several weeks of dual therapy. TVR therapy acts on the same guidelines and schedule of treatment as BOC therapy uses.

On-treatment virological response

Depending on on-treatment virological response, kind of baseline, a low versus high, for HCV RNA level might be used for the guidance of decision-making for treatment during dual therapy is going on. Still there is no best discerning value of HCV RNA level has been agreed upon, while the range lies from 400000-800000 IU/mL[73-80]. If decrement in the value of HCV RNA is < $log10^2$ IU/mL with dual therapy, then the treatment should be stopped at 12th week and the rate of SVR is < 2% if treatment remains continued. At 24th week if the level of HCV RNA is still detectable then there is only 1%-3% chance of

SVR so the therapy should be discontinued[73,74,79,81]. When detection tests were less sensitive than modern assays, the futility rule was defined at that time. By using modern assays, treatment should not be discontinued for people showing undetectable RNA. In BOC triple therapy, SPRINT-2 study has been analyzed order to derive futility rules. If level of RNA is greater than 100 IU/mL at 12th week of treatment then all medications should be stopped even also if that is detectable at 24th week and also when there is a viral breakthrough (reappearance of HCV RNA during the course of treatment post to virological response). For TVR triple therapy, ADVANCE database has been used to derive stopping rules. It its case the drugs should be discontinued at 4th week of treatment if RNA level is greater than 1000 IU/mL and also when there is breakthrough.

Dual therapy guided by virological response

The infected persons showing an undetectable HCV RNA and decrease of > $log 10^2$ in the 12th week can be grouped into three as per virological response: First is the RVR that defines an undetectable HCV RNA in the 4th week of therapy. Second is early virological response (EVR) or cEVR (complete EVR) that describes undetectable HCV RNA in the 12th week of therapy. Third that defines decrease of > $log10^2$ with HCV RNA detectable at week 12 and undetectable in the 24th week is thus referred to as delayed virological response (DVR) or pEVR (partial virological response).

Evaluation of safety post-treatment

It is necessary to monitor the patients after treatment so to avoid side effects and if they have encountered with them then they should be controlled. For instance, after peg-interferon-α injections sometimes flu-like symptoms start appearing. So paracetamol should be prescribed to control the symptoms. Other clinical side effects like irritability, allergic reactions, and severe fatigue should be checked with routine visit. Dermatological and hematological adverse reactions should be assessed both during and after treatment. Levels of thyroid stimulating hormone (TSH) should be quantified during treatment after every 12 wk[82]. Even the dosage of peg-IFN-α should be decreased when concentration of platelets and neutrophils drops lower than 50000/mm^3 and 750/mm^3 respectively. Even the medication can be stopped when there is a remarkable decrease in the amount of platelets and neutrophils, *i.e.,* less than 25000/mm^3 and 500/mm^3 respectively and severe depression. Treatment should be discontinued in case if any bacterial infection is detected during treatment[73,83,74].

Adjustment of cofactors

Cofactors such as body weight, lipids, alcohol and growth regulators should be adjusted. High value of body mass index (BMI) badly affects the response to

treatment (PegIFN/RBV)[84]. It should be suggested to reduce body weight before starting treatment. It can be co-related with the concentration of lipids as high body weight can be due to high level of lipids. The life cycle of hepatitis C virus is associated with the metabolism of lipids. Consumption of alcohol can have a severe impact on treatment actually it affects adherence to treatment[85]. Growth regulators limit the need for reducing drug dosage, *e.g.,* EPO (recombinant erythropoietin) improves the levels of hemoglobin by avoiding the decrease in the dose of ribavirin. Then a metabolic syndrome, type 2 diabetes and resistance to insulin, enhance the development of liver disease thus also can lead to hepato-cellular carcinoma. Furthermore, they may also be responsible for dampening the response to dual therapy[86].

Transplantation of liver
In case when liver is at the last stage of disease then only one treatment, liver transplantation (LT), is left. But there are still chances of recurrence of hepatitis C[87]. In order to achieve SVR, antiviral therapy should be initiated as quickly as possible in the patients who are suffering from acute liver disease but their treatment is probable before transplanting liver[88].

Treating the unusual groups
HIV-HCV co-infection: The rate of development of liver inflammation increases when HCV patients are co-infected with HIV. It happens especially in patients with weaken immune system and a low cell count of CD4-positive cells. These patients should be given antiretroviral treatment before starting anti-HCV treatment[89]. The use of these antiviral drugs, stavudine, zidovudine and didanosine should be evaded during PegIFN/ribavirin therapy[90]. Non-invasive techniques or biopsy should be used to measure severity of liver disorder.

Treatment of hemodialysis patients
As the risk of HCV infection development is enhanced in the patients undergoing hemodialysis. Actually this infection becomes the major cause of death in these patients. These patients require highly specialized antiviral therapy so ensure the survival of the person.

A new era of direct acting anti-HCV agents
Though the current standard therapy for chronic HCV infected patients is pegylated interferon-α in combination with ribavirin, however, a number of side effects of the current standard therapy usually make adherence to treatment difficult and reducing an SVR[91].

A new era of direct acting anti-viral (DAA) defined as agents/compounds that interfere with specific steps in the HCV life cycle through a direct interaction with the HCV encoded polyprotein and its cleavage products has emerged. To elevate SVR, DAAs are given in combination to the existing standard of care (pegylated interferon and ribavirin) for chronic hepatitis C infected patients with genotype 1 infection. Of these DAAs, 2 protease inhibitors, telaprevir and boceprevir, are in use for HCV treatment since 2011[92]. Other DAAs recently in use are TMC-435, vaniprevir, BI-201335, BMS-650032, Mericitabine (RG-7128), Tegobuvir (GS-9190), Daclatasvir (BMS-790052) and danoprevir. However, treatment outcome shows an overall efficacy rates between 70%-90% in no improvement in side effects.

Recently several other anti-viral agents are developed that directly target various stages of HCV replication and are proved very effective interferon-free therapy for HCV-infected patients[93]. Of these, the most important is oral nucleotide analogue "Sofosbuvir" (also known as GS-7977) that is an inhibitor of the HCV nonstructural protein 5B (NS5B) RNA-dependent RNA polymerase enzyme and is very effective against HCV. Sofosbuvir was recently approved for use in combination with ribavirin and/or pegylated interferon for chronic HCV infection, depending on the genotype. The recommended regimens and duration of therapy: (1) for genotype 1 or 4 chronic hepatitis C: Sofosbuvir, peginterferon alfa, and ribavirin for 12 wk; (2) for genotype 2: Sofosbuvir and ribavirin for 12 wk; (3) for genotype 3: Sofosbuvir and ribavirin for 24 wk; and (4) for hepatocellular carcinoma awaiting liver transplantation: Sofosbuvir and ribavirin for up to 48 wk or until liver transplantation (whichever occurs first). Sofosbuvir and ribavirin oral therapy is very effective against genotypes 2 and 3 HCV infections[94].

Renal transplant recipients infected with HCV
Recipients of kidney transplant when become infected with Hepatitis C might develop fibrosis of liver. If transplant is improperly carried out then the patients can become HCV positive and can be threatening for the life of the person[95]. The measurement of fibrosis should be ensured in these renal-transplant patients having HCV in serum.

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