Journal of Taibah University Medical Sciences (2015) 10(2), 132–139

Taibah University

Journal of Taibah University Medical Sciences

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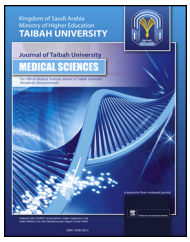

Review Article

Concise review on the insight of hepatitis C

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Received 11 January 2014; revised 5 August 2014; accepted 6 August 2014; Available online 6 January 2015

الملخص

التهاب الكبد "ج" هو مرض يصيب الكبد، وينجم عن التهاب الكبد بفيروس الكبد "ج". ونظرا لتأثيره واسع النطاق على السكان، هناك زيادة مستمرة في العوامل العلاجية الجديدة لعلاجه والحد من التهاب الكبد الفيروسي "ج". لذلك يعتبر التهاب الكبد الفيروسي "ج" في الوقت الحاضر عبء عالمي في جميع أنحاء العالم. ويعتمد التقدم في التدخلات العلاجية والنتائج السريرية على جينوم التهاب الكبد الفيروسي "ج" وطبيعته المتنوعة، وطبيعة المرض، والعوامل الغذائية، والعوامل الاجتماعية، والاقتصادية والبيئية. تم التركيز في هذا الاستعراض بشكل رئيس على جينوم التهاب الكبد الفيروسي "ج"، وتاريخ المرض والنتائج السريرية منذ اكتشافه إلى الأبحاث الحديثة. روجعت في هذه المقالة المعلومات المنشورة من عام ١٩٩٧م إلى ٢٠١٤م. وكانت المواضيع ذات الاهتمام الرئيس هي التهاب الكبد الفيروسي "ج" ذو الطبيعة المتنوعة، والتطورات الحديثة في علاج التهاب الكبد "ج" المزمن. واستخدمت المصطلحات "التهاب الكبد الفيروسي ج"، "التطورات الحديثة لعلاج التهاب الكبد الفيروسي ج"، "التهاب الكبد الفيروسي والمناعة واللقاح"، أو "علاج مضادات الفيروسات" للبحث في قواعد المعلومات. وأدرجت جميع الدراسات التي تم تحديدها وفقا لوصف العنوان الفرعي.

الكلمات المفتاحية: التهاب الكبد ج; التهاب الكبد الفيروسي ج; التدخل العلاجي; الالتهاب

Abstract

Hepatitis C is the disease of liver caused by hepatitis C virus (HCV). Due to its widespread impact on human population, there is continued surge for new therapeutic agents to treat and reduce HCV. Hence, nowadays HCV is considered as global burden throughout the world. Advancements in therapeutic invention and clinical outcomes are dependent on HCV genome and diversity in nature, pathogenesis, dietary factors, social, economic and environmental factors. In this review we have focused mainly on HCV genome, its history and clinical outcomes from its discovery to present day research.

In this article the authors have reviewed the published data from year 1997 to 2014. The topics of main concerns were hepatitis C with diverse nature and recent advances in the treatment of chronic hepatitis C. The authors used MeSH terms "Hepatitis C Virus (HCV)", recent advances in the treatment of "HCV", "HCV and Immunity", "vaccination", or "Interferon therapy" to search the PubMed database.

All relevant studies identified were included and are described according to the subheadings. Recent advancement in molecular biology and experimental techniques has opened new insights into the pathophysiology of HCV which is helping in combating this life threatening disease.

Although the response to current treatment regimen for HCV is improved however complete recovery from the disease is still a challenge which requires more extensive

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Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.jtumed.2014.08.004

studies from basic science research to large multicenter clinical trials.

Keywords: Hepatitis C; HCV; Inflammation; Therapeutic invention

Introduction

Hepatitis C is a complex disease of liver. Due its widespread nature and global burden, this disease has always attracted attention for insight into its causative agent, hepatitis C virus (HCV), and for the development of new therapeutic approaches. Even after twenty four years of its discovery, HCV continues to be a major cause of concern and a huge burden on public health systems worldwide. WHO estimates that minimum of 3% of the world's population is chronically infected with HCV.[1,2] As cited in various research articles that HCV has caused massive impact on public health, and around 170 million people in the world are infected with HCV, with an estimated 3 to 4 million new infections global per year.[3] It causes infection in two phases, first involve acute attack that last for few weeks and if untreated then it may persist for long time that is termed as chronic hepatitis C. This chronic infection may often lead to chronic liver disease (CLD) that may ultimately lead to even hepatic failure.[4]

This review covers the basic structure of HCV and new insights in development of novel treatment method and strategies to counteract this aggressive disease. Persistent virus infection with HCV often leads to cirrhosis and hepatocellular carcinoma (HCC). As cited in literature that it is difficult to combat the infection due to diverse nature of this virus. The recent research is directed at genome level and has discovered many antiviral therapies providing evidences about the new antiviral drugs against HCV. Presently in market, available treatment option is a long-acting pegylated-interferon-alpha, given in combination with nucleoside analog ribavirin, which is quite effective but due to adverse reaction, relapses, and poor health condition many of the patients resort to discontinue the therapy.[5] Also various traditional herbal medicines are available in the market practiced by Unani or Ayurvedic or traditional Chinese medicine (TCM) practitioner in Pakistan, India, and China. These medicines are alternative treatment option for this chronic disease, but due to lack of advance research on scientific basis, these medicines are not widely utilized as compared to allopathic medicine. It is strongly suggested that these traditional medicines should be justified on scientific grounds and comparative clinical trials on large scale are direly needed, which might be fruitful in identifying curative medicinal herbs. This may create alternate options for researches to reduce the global burden of diseases by sorting natural source of treatment. Molecular studies of HCV had started with the successful cloning of its genome in 1989. A major milestone was achieved with the recent development of a robust cell culture system for HCV propagation. HCV proteins assemble and form replication complexes on modified host membranes, called as membranous webs. Even though HCV is detected and targeted by host immune mechanisms, it establishes and maintains a life-long persistent infection. It evolved different strategies for its survival in hostile cellular environments. The HCV population is well known to rapidly change during the course of natural infection, thereby, escaping the immune surveillance. For survival it may select variants that are resistant to antiviral drugs. The extremely complex nature of this virus lifecycle had left complication for discovery of new therapies. A complete understanding of the functional roles played by the HCV proteins during its lifecycle is vital for developing a successful cure.

History of hepatitis C

The HCV belongs to the most successful of all persistent human viruses. With a compact RNA genome thought to encode only 11 proteins, HCV persists in up to 70% of those infected by successfully undermining virus-specific immunity while leaving host defenses to other infectious agents intact. An estimated 170 million individuals are infected worldwide, and approximately 38,000 new infections occur annually in the United States alone.[6] Twenty percent of persistently infected individuals will develop liver cirrhosis, and approximately 2.5% progress into hepatocellular carcinoma (HCC). Furthermore, current anti-viral therapy is effective in only 50−60% of all the patients treated but is much expensive, and at same time it exerts side effects.[7] The precise understanding of adaptive immunity response against the virus is crucial for the design of effective strategies in order to control HCV both in the infected and healthy individuals as well for prevention from this infection. The outcome of HCV infection is determined within six months of exposure to the virus. Acute infection is often unrecognized because symptoms are usually mild or absent. After first attack that is called acute, it may turn into chronic infection which if not treated at earlier stage, may initiate innate immunity against this virus.[8] It is reported that there is strong relation between adaptive immunity and HCV. Ge et al. described that there is involvement of humoral immunity in natural clearance of this virus and treatment response of interferon as well.[9] It has been described that there is involvement of antiviral CD4+ helper cells and the CD8+ cytotoxic T cells in acute phase. As a corollary to this response, memory cells are produced in the body that helps to reduce the risk of re-exposure of this infection. It is reported that there is significant relation of polymorphism of 3 kb upstream of IL28B with pegylated interferon and ribavirin for treating the chronic hepatitis C due to genotype 1. This shows that gene product associated with natural clearance of this infection is involved in innate immune response against HCV. It was experimentally proved *in vitro* and *in vivo* that interferon therapy shows strong antiviral activity against genotype 1.[10,11] The IFN-λ proteins, encoded by the *IL28A/B* and *IL29* genes, were identified in 2003.[12,13] The specific target of IFN to study underlying gene response (i.e. IL28B) and cell signaling are potential target for development of new antiviral drugs.[9] For novel antiviral therapies it is necessary to target structural proteins variants that are vital for viral

replication and even survival i.e. NS3-protease and NS5B RNA-dependent RNA polymerase.[14,15] It is imperative to explore the relationship between functional and structural equivalence of this virus and to determine the specific location and strain of genome that may help in vaccine development against HCV.[16]

Virology

Hepatitis C is a single stranded RNA virus that belongs to a family flaviviridae. In 1989 HCV was identified as major causative agent of hepatitis C.[17] HCV is a small (~55–65 nm), spherical in shape, enveloped, hepatotropic RNA virus that causes acute and chronic hepatitis in human.[18] It encodes a protein chain of 3010 amino acids and contains structural and non-structural proteins.[19,20] HCV consist of a 9.6-kb single-stranded positive sense RNA genome with a 5′ untranslated region (UTR) that functions as an internal ribosome entry site, a single long open reading frame encoding a polyprotein of approximately 3000 amino acids (aa) and a 3′ UTR. This polypeptide is post-translationally cleaved by host cell peptidases (proteases) to yield three structural proteins named E1, E2, core, p7 and by viral proteases, which generate the six non-structural proteins named as NS2, NS3, NS4a, NS4b, N4a and NS5B.[21–25] By analogy to related positive-strand RNA viruses, replication occurs by means of a negative-strand RNA intermediate and is catalyzed by the NS proteins, which form a cytoplasmic membrane-associated replicase complex.[26]

Its various genotypes (GT) identified are 1 2, 3, 4, 5, 6, and 7 that shows the distribution of this virus worldwide.[27,28] Most common genotypes are GT 1–4.The seven confirmed genotypes comprise 67 subtypes, 20 provisionally assigned subtypes, and 21 unassigned subtypes. The natural course of infection seems to vary with the genotype; some are associated with a higher chance of acute infection being cleared i.e. GT3 is more likely to clear compared with GT1).[29,30] In fact, HCV circulated in infected individuals as a population of diverse but closely related variants termed as "quasispecies". The incubation period of HCV is 6–8 weeks. It is referred as silent disease or silent killer" because it remains asymptomatic and undetected at early stage rendering the early treatment of HCV infection difficult. HCV does not resolve in majority of cases and later on leads to greater complication that would damage liver at large. During natural course of infection, neutralizing antibodies (that mask the antigenicity) are produced that is why it escape surveillance.[31] This disease has no constant outcomes; it can vary from mild to severe and may cause fibrosis. The flow chart that is given below in Figure 1 describes the stages of liver damage from acute manifestation of HCV till HCC. This acute type may resolve in about 20% of cases while remaining 80% of cases are converted into chronic clinical presentation. In the chronic condition it presents with three different stages i.e. mild, moderate and severe. If it is not treated at early stage then the outcome of all these is liver cirrhosis that may lead to either HCC. The process of disease in which

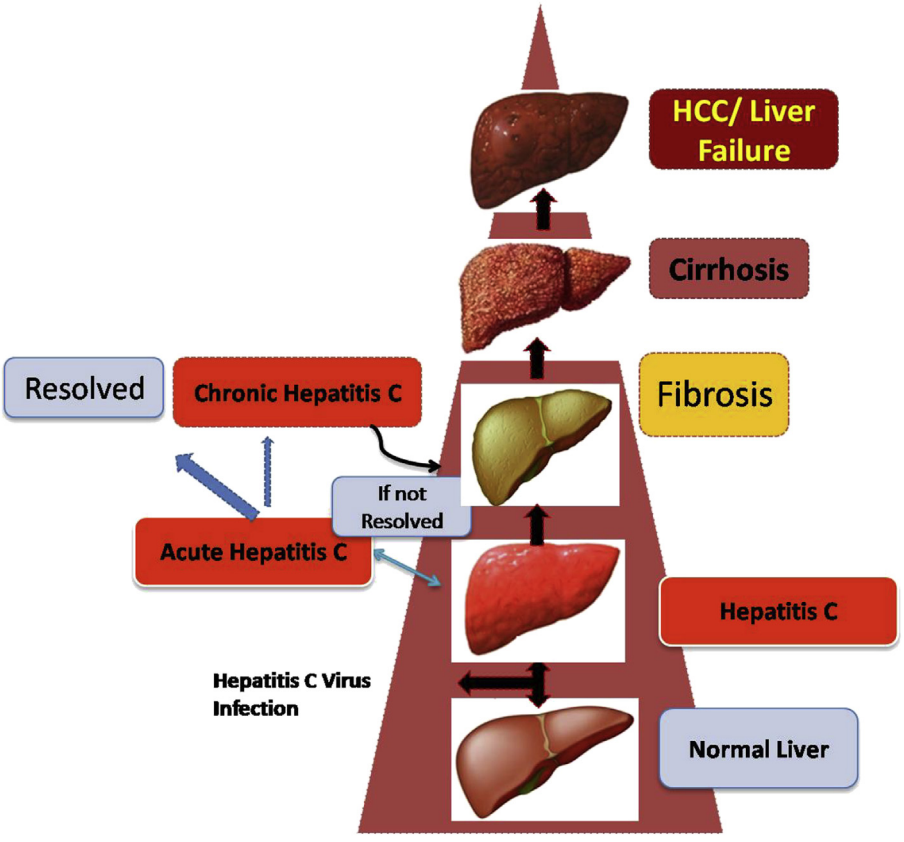

Figure 1: Stages of liver damage. This figure shows how normal healthy liver develop into hepatitis C, fibrosis, cirrhosis and even hepatocellular carcinoma or liver failure.

liver is affected and being diseased from healthy liver to reach cirrhosis or HCC is given in Figure 2.

Mode of transmission

Parenteral route is primarily source of spread of hepatitis C. Those who are at higher risk for getting hepatitis C are intravenous drug users (90%), and blood transfusion, blood products such as hemophiliacs and during surgical procedures whereas 0.4–3% transmission occur by sexual or intimate contact much less than hepatitis B (HBV).[32] Approximately 3–5% transmission occurs through mothers infected with HCV to newborn infants. This transmission of infection from mother to infant is mainly due to vertical transmission and there are no or very rare chances from breastfeeding because this virus become inactivated in the digestive tract of infant.[33–35] Through needle stick injury there is 5% chances of transmission of HCV.[36] In saliva and breast milk, HCV has been found but not documented to spread through breast milk.[37] Needle stick open wounds at work may be affected with HCV or transmission of HCV occurs through some other potential source which may include the sharing or contact sports and other activities i.e. slam dancing which may relate to blood exposure.[27,38,39] Through improper sterilization techniques, the HCV can spread from one person to another by tattooing dyes, stylets, inkpots, and piercing etc. Toothbrushes, razors, cuticle scissors or excessive visits to barbers can be the source of transmission or spread of HCV to healthy person by sharing these with infected individuals.[40]

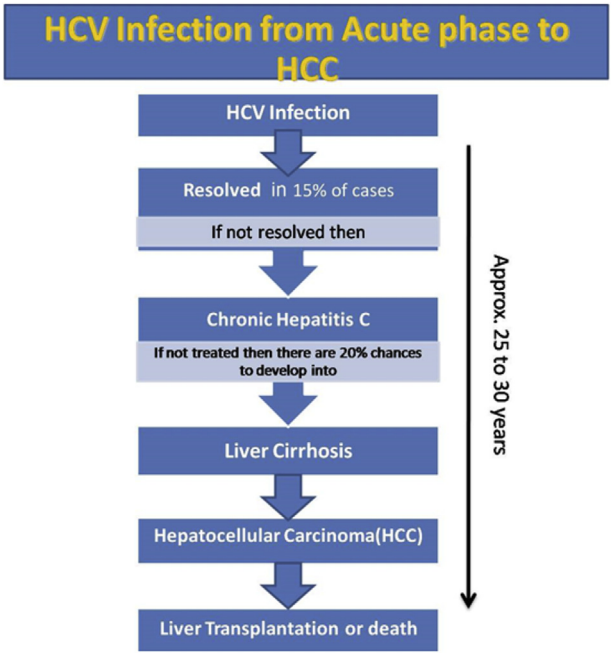

Figure 2: History of HCV infection from acute phase to HCC; Exposure of HCV may lead to acute infection and if not treated then it may lead to chronic infection in 85% of cases. From chronic hepatitis to cirrhosis it may take approximately 20 years to cause cirrhosis or end stage liver disease.
(Reference: Di Bisceglie A, et al. *Hepatology*. 2000; 31:1014–1018).

Natural course of pathogenesis

After the infection with HCV, the virus can be detected within 1–4 weeks *prior to* the increase in liver enzymes.[41] The pathogenicity of liver is so complex that it cannot be explained clearly even after 24 years of research from its discovery. In 25% of cases, the jaundice may appear in HCV positive patients but mostly remains symptomless that are why it is not identified at very early stage.[42] Chronic infection will develop in more than 85% of HCV positive patients as shown in Figure 2. In most of the cases, this infection is cleared automatically by powerful immune system i.e. natural or cell mediated immunity. In some cases with weak immune system, antibodies are not produced against HCV and ultimately are not detected by either polymerase chain reaction (PCR) for HCV RNA or anti-HCV antibodies test.[43]

Inbuilt immunity: The invasion of HCV occur mostly when the natural immunity of human being become weak and concentration of invading agent become excessive or more copies are formed. When the natural immunity becomes weak or decreased due to any impeding factors, there are increased chances of invasion of HCV causing severe disturbance in structure and function of liver. There is also association of autoimmunity and cryoglobulinemia with hepatitis C.[44,45] Some of the evidence proved that cell mediated immunity (CD8+) have a main role in HCV pathogenesis and killing of HCV infected hepatocytes.[41]

Humoral immunity: Hepatitis C infection is not cleared spontaneously in 85% of infected individuals. When anti-HCV antibodies are positive in HCV persistent infection, it means these antibodies are not enough to clear the infection in chronic infected patients. To fight against HCV, the humoral immunity plays an important role to assist in direct neutralization of cell free virions. The humoral immunity has very limited role in the cell to clear off the HCV completely.[46]

Cell mediated immunity: The host shows response against HCV infection by helper T-cells and T-cytotoxic cells. In resolution of HCV infection, T-helper and T-cytotoxic cells plays a major role in providing immunity at cellular level and offer a very powerful defense mechanism. The activated T-cells can kill HCV hidden in cells of liver and can make hepatocytes and kupffer cells so strong that they cannot exhibit any injurious or harmful event in liver.[47]

Clinical presentation of hepatitis C without cirrhosis

Although HCV affects liver but it exert effects on almost every organ as well as whole body. In 14% of infected patients with HCV, signs and symptoms of hepatitis C are positive while 86% of the infected patients remains asymptomatic.[48] HCV has deteriorating effect on whole body systems which produce exacerbating symptoms mostly include fatigue, fever, epigastric pain, burning micturation, heart burn, burning palm and sole, chills, fluid retention and muscle pain.[49] These may also include weakness and generalized itching. There is also involvement of systemic (gastrointestinal), cognitive, mood and nervous systems in this disease to display the relative intensity of symptoms. It may also include hypoglycemia, hyperglycemia, chest pain, menstrual changes, palpitations and also sexual anomalies.[49]

Investigation

Gold standard to investigate hepatitis C starts with serological blood test that is specifically designed for the detection of HCV. These anti-HCV antibodies may be diagnosed in nearly 80% of cases within 15 weeks of exposure. In general, anti-HCV antibodies have a very strong positive prediction value after exposure. However, in some of the cases these are not detected and missed in patients who have not developed these antibodies or the level of antibodies is insufficient to be diagnosed. Those patients who are immunocompromised can never develop antibodies to HCV and anti-HCV test could not be ascertained as positive. For this possibility of molecular RNA testing should be done even when anti-HCV test is negative and the serum alanine aminotransferases and alkaline phosphatase are found raised.[50]

Management

The management of hepatitis C includes different ways to control and combat this disease. The major line of treatment may include; to treat actual cause with immunostimulants, antioxidants, blood purifiers, diuretics, hepatoprotectives and to change the internal environment in which HCV cannot survive.

New insights and prospective

The effectiveness of interferon alpha 2b and ribavirin combination therapy in the treatment of naïve chronic hepatitis C patients have been cited in the literature.[51] Gary et al. described that combination of seven botanical drugs Sho-Sai-Ko or xiao-chai-hu-lang in China utilizes for the treatment of hepatitis C have improved the liver pathogenicity in selected hepatitis C patients.[52] We also conducted similar study on Hepcinal, a coded herbal formulation, comprised of five botanical drug components (namely: *Silybum marianum*, *Picrorrhiza kurroa*, *Glycyrrhizaglabra*, *Tamarix gallica*, *Rosa damascena*) have shown marked clinical improvements in the signs and symptoms of this disease (Unpublished data). It is recently reported that antibodies produced from different genotypes of HCV may neutralize the pseudoparticles to control the infection naturally. Pseudoparticles are genetically tagged HCV particles harboring unmodified E1 and E2 glycoproteins. High infectivity of these particles allowed the precise investigation of HCV E1 and E2 glycoproteins and their potential receptors in cell entry, HCV host-range, and neutralization by antibodies from HCV patient sera.[53,54] This suggests that a broad cross-neutralizing antibody to HCV may exist and could be exploited in vaccine strategies. This concept of neutralizing the antibodies in pseudoparticles infectivity may explore that acute infection in some cases may recover automatically during this neutralizing process of inbuilt immune activity.[55] The relative roles of humoral and cellular immunity in recovery remain unclear. Continuous effort from several years helped to explain the sophisticated battle that is initiated in the infected host. The RNA virus cannot integrate into the host genome and it may develop the mechanisms to persist and to evade the host's innate and the adaptive immune mechanisms. The virus has the ability to inhibit the induction of the type-1 interferon, may inhibit the NK cells and readily produces escape mutants to cytotoxic T lymphocyte and neutralizing antibodies directed to the amino-terminal region of gpE2.[56–65] It may also inhibit the binding of the virion-neutralizing antibodies by masking with the lipoproteins.[66] Bowen and Walker found that cell mediated immunity play critical role for preventing the HCV persistence.[8] They concluded by their observation that immunity has major role in the prevention from different genotypes and open new hopes for development of safe and effective vaccine. They also found close relation between CD4+ TH cells and HCV infection. These cells may be the best candidate in future study to cure and prevents the progression of this disease.[8] The next generation of direct acting antivirals and the first interferon-free regimen for the treatment of HCV have recently been approved. Over the next one or two years, several new agents (protease inhibitors and NS5B inhibitors) and classes (NS5A inhibitors) of direct acting antivirals are likely to be licensed. Newer protease inhibitors will broaden the range of peginterferon–ribavirin containing regimens available, but most importantly the options available for interferon-free regimens will grow. The combinations of sofosbuvir–ledipasvir, sofosbuvir–GS-9669, and the regimens of ABT-450–ritonavir–ombitasvir–dasabuvir, and daclatasvir–asunaprevir–BMS-791325 are expected to be approved in the near future. These interferon-free regimens could enable many patients with HCV (even those with cirrhosis and those who have not responded to previous protease inhibitor based treatment) to be cured with an oral course of antivirals without the use of interferon and its associated side effects. Despite this optimism, challenges remain, particularly in the treatment of patients with HCV GT3, cirrhosis, hepatic decompensation, and liver transplantation.[29,67]

Ralston et al. reported on vaccine development and found promising findings using recombinant technology.[68] For vaccine development they involved HCV recombinant glycoproteins i.e. gpE1 and gpE2 derived from mammalian cells, that can form a non-disulphide link to resemble the pre-virion envelope structure. They found that vaccinated experimental animals (anti-gpE1/gpE2 antibody titres) were protected from the infection when vaccinated.[69] They had used the sensitive RT–PCR assays to analyze viraemia from blood or liver samples and found no viraemia in these experimental animals. They suggested that this response may be due to the sterilizing immunity correlated directly with anti-gpE2 antibody titers that may prevent the binding of gpE2/virus itself to the CD81 cells and it is important receptor for HCV and for the cell entry of the lentiviral/HCV pseudoparticles.[52,70–72] Therefore, they also found that lower-responding animals were infected again and mainly they bypass the acute infection which in the humans, can be associated with the chronic liver disease.[67,73–76] They concluded that there is low rate of carriers in vaccinated as compared with the control group that was not immunized.[67,73] It was inferred in many research studies that recovery from the acute HCV infection correlate with the cellular immunity against this virus, and for the development of vaccine it may involve both humoral and cell mediated immunity against the HCV.[77] Arora et al. demonstrated high rates of cure for the HCV treatment delivered through different model in community-based

study.[78] They found comparable sustained virologic response (SVR) rates in overall response and also in genotype 1.[79–81]

Outlook and future directions

It is clearly an exciting time in HCV research, and rapid progress should be made now due to availability of complete cell culture systems. It is expected that *in vivo* and *in vitro* study should be conducted by using human HCV and thereafter monitor its replication and then treat by using targeted medicine with and without diet restriction. Massive and descriptive studies should be conducted from baseline to investigate exact nature and replication processes of virus and correlation of immunity or nerve impulses should also be analyzed. Early enhancement in development strategies against this virus is necessary to overcome its circumstances to remain in control, reduce danger and threat to lead healthy life. Many promising agents are in the pipeline to bring about evidence based therapies.

The improved response rates and treatment duration of hepatitis C with both allopathic and traditional medicine are delineated in literature; however, limitation still exists for its total curative function. Research in hepatitis C field should move from small, single center and go beyond large multi-center strategies as more and more information is available from genetics to immunology perspective. However, more stress is needed to execute surveillance for cirrhosis and hepatitis C for standard care.

Author contribution

All the authors have contributed equally in writing, literature search and formatting of this review.

Conflict of interest

The authors have no conflict of interest to declare.

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