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Review Paper

Hepatitis C virus infection: A review of the current and future aspects and concerns in Pakistan

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Hepatitis C virus (HCV) is the major etiological agent of hepatitis. It infects 200 million people worldwide and 85% of them could develop chronic hepatitis, liver function failure or hepatocellular carcinoma. Hepatitis C is rapidly emerging as a major health problem in developing countries like Pakistan with prevalence rate of 10% and genotype 3a is the most prevalent. Here, approximately 80% of infections proceed to chronic infection and infected blood is the primary route of spread. In Pakistan, about 75% of patents do not receive standard anti HCV therapy (Interferon + Ribavirin) and of the 25% that do receive such treatment, the SVR rate is 60 - 70%. This review is designed to cover the information about the status of HCV in Pakistan with major focus on its prevalence, genotypes, current diagnostic assays, available therapies and treatment outcomes. The present review further emphasizes the need to uncover exact HCV prevalence rate in the country, to develop diagnostic assays based on local genotype, to understand the interaction between HCV genotype 3a genes and cell line genes responsible HCV pathogenesis. In addition, this review discusses the need for the generation of infectious pseudo particle of HCV as a potential vaccine, to investigate DNA base vaccine, or siRNA-based anti HCV approaches for our local genotypes.

Key words: Hepatitis C, prevalence, genotypes, treatment, Pakistan.

INTRODUCTION

Hepatitis C Virus was isolated in 1989; a member of Flaviviridae and is a major pathogen of hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (Kato, 2001; Bhandari and Wright, 1995; Shepard et al., 2005; Giannini and Brechot, 2003). It is estimated that 3.3% of the population globally (lower in Europe 1.03% and highest in Africa 5.3%) and 10% of the Pakistani population is chronically infected with this viral pathogen (Hepatitis C, 2009, Raza et al., 2007; Farhana et al., 2009; Idrees and Riazuddin, 2009). In industrial countries the HCV accounts for 20% of acute and 70% of chronic cases of hepatitis (Farhana et al., 2009).

Major HCV infections lead to chronic hepatitis, which results in progressive fibrosis ultimately resulting in cirrhosis, liver failure and an increased risk of hepatocellular carcinoma (Shepard et al., 2005; Giannini and Brechot, 2003; Freeman et al., 2001; Jacobs et al., 2005). According to Farhana et al. (2009), 40% of HCV infections end stage cirrhosis, 60% hepatocellular carcinoma and 30% of liver transplantation (Farhana et al., 2009).

Pakistan is the sixth most populous country in the world with total estimated population of 170 million and 803,940 Km^2 land area (Idrees and Riazuddin, 2008). It is situated in the Western part of the Indian subcontinent, with Afghanistan and Iran on the west, India on the east and the Arabian Sea on the south. It is a federation of 4 provinces (Balochistan, North West Frontier Province, Punjab and Sindh), a capital territory and federally administered tribal areas. The present review summarizing the current information available about prevalence, most prevalent genotype, pathogenesis, modes of transmission, diagnostic assays, treatment response rate in Pakistan and also emphasizes for the further needs to

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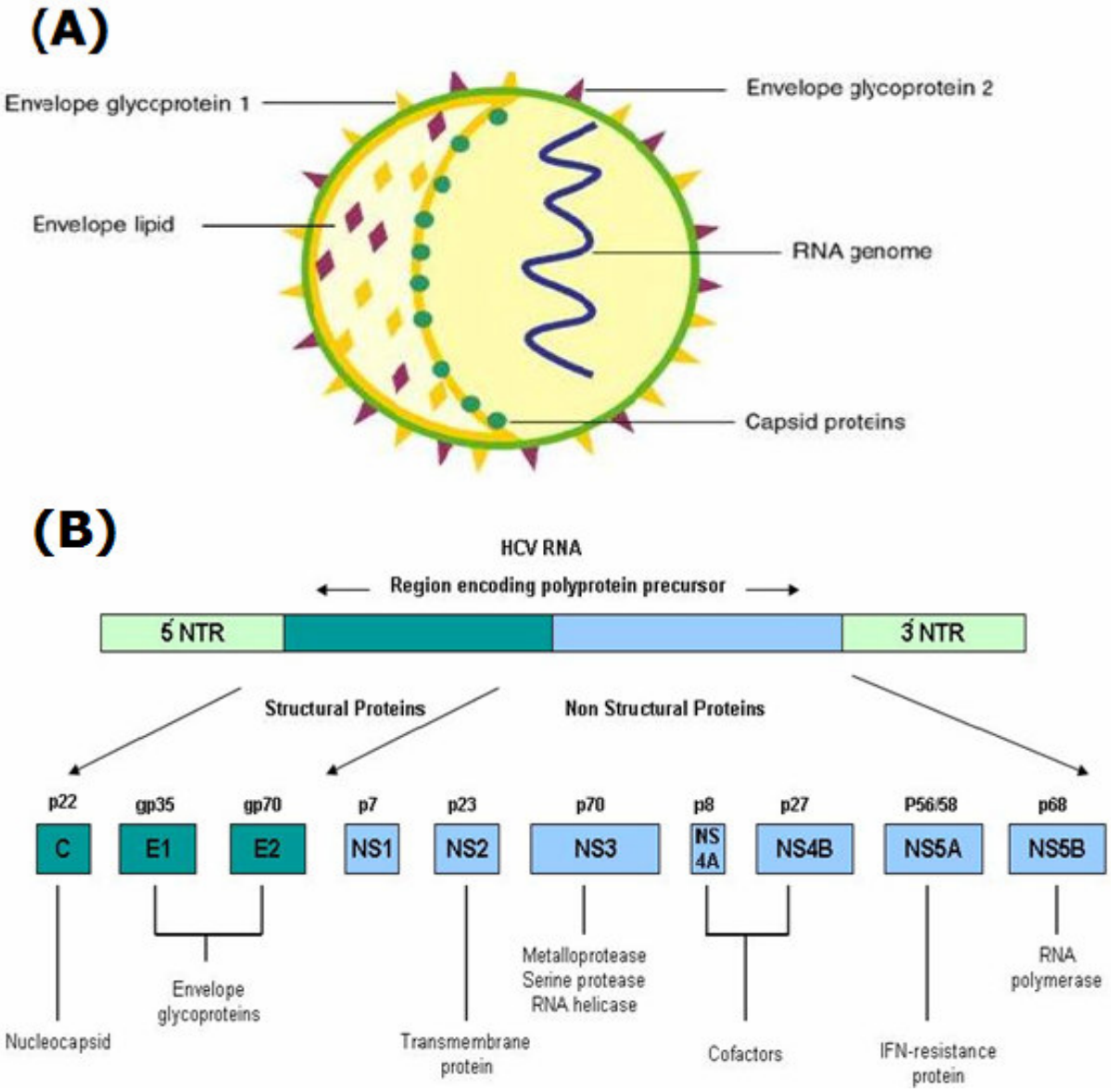

Figure 1. (A). Model structure of HCV: The left-hand side of the illustration shows the viral surface of envelope lipids and glycoproteins; the right-hand side shows the RNA genome encased by capsid proteins. **(B). HCV genome organization:** Proteins encoded by the HCV genome. HCV is formed by an enveloped particle harbouring a plus-strand RNA of 9.6 kb. The genome carries a long open-reading frame (ORF) encoding a polyprotein precursor of 3010 amino acids. Translation of the HCV ORF is directed via a 340 nucleotide long 5' nontranslated region (NTR) functioning as an internal ribosome entry site; it permits the direct binding of ribosomes in close proximity to the start codon of the ORF. The HCV polyprotein is cleaved co- and post-translationally by cellular and viral proteases to produce 10 mature proteins as indicated, including the four structural proteins: core, E1, E2, and p7 and the 6 non-structural proteins, NS2, NS3, NS4a, NS4b, N4a, NS5B. Putative functions of the cleavage products are shown (Adopted from NET).

eradicate this from the country.

Hepatitis C virus (HCV)

HCV is an enveloped virus consisting of a single positive-sense strand RNA genome of about 9.6 kb, which encodes a polyprotein of 3010 amino acids processed by cellular- and virally-encoded proteases into 4 structural proteins (core, p7 and the envelope glycoprotein, E1 and E2) and 6 nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Figure 1) (Dubuisson, 2007; Choo et al., 1991; Reed and Rice, 2000; De Francesco and Steinkuhler, 2000; Lin et al., 1994; Posta et al., 2008). Based on genome sequence similarity, international

standardization of nomenclature recently classified HCV into 6 major genotypes (1 - 6) and more than 70 sub genotypes which differ 30 and 15% at the nucleotide level, respectively. Their prevalence and distribution are linked to geographical location and mode of transmission. (Simmonds, 2004; Roman et al., 2008; Simmonds et al., 1994).

Prevalence and genotypes of HCV in Pakistan

Hepatitis C is rapidly emerging as a major health problem in developing countries including Pakistan (Raza et al., 2007; Khan et al., 2008). It is estimated recently that 200 million individuals are infected with HCV worldwide including approximately 17 million in Pakistan (Narendra et al., 2004; Idrees and Riazuddin, 2008). Prevalence of HCV may be different in different regions and various groups of the same community (Idrees et al., 2008). Hospital-based studies revealed prevalence rates of 5.31% (Islamabad), 2.45% (Rawalpindi), 4.06% (Multan), 20.89% (Faisalabad), 4-6% (Karachi), 9% (Mardan), 5% (Buner, NWFP) and 25.7% (Northern Areas) (Farhana et al., 2009; Chaudhary et al., 2007; Jehangir et al., 2006; Hashmie et al., 1999; Kazmi et al., 1997; Khan et al., 2004; Muhammad and Jan, 2005; Tariq et al., 1999). Slightly higher prevalence of HCV was recorded in the earth quake effect areas of Pakistan in 2005 (Khan et al., 2008). A recent study showed that in Pakistan more than 90% of HCV positive subjects were unaware of HCV infection in general (Idrees et al., 2008).

Previously, 3351 serum samples with viral load > 500 IU/ml were successfully typed by genotype-specific genotyping assay. Out of which 2165 genotyped patients belonged to Punjab region, 823 patients to N.W.F.P., 239 to Sindh and 124 patients were from Balochistan (Idrees and Riazuddin, 2008). It has been observed that the prevalence of HCV increases with age which may be due to increasing exposure to risk factors (Idrees et al., 2008).

Recently an improved genotyping method for detection of HCV genotypes and subtypes in Pakistan was developed that is based on entire core region and a part from 5 non-coding region (5'NCR) with specific primer, the current system is reliable, sensitive, specific and economical for genotyping assay of for Pakistani HCV isolates (Idrees, 2008). According to a recent study, in Pakistan the observed genotypic distribution were 3a (49.05%), 3b (17.66%), 1a (8.35%), 2a (7.52%), 1b (3.01%), 4 (1.49%), 3c (0.75%), 2b (0.80%), 5a (0.18%), 1c (0.15%), 6a (0.12%), 2c (0.09%) and mixed infection (4.80%). During this study genotype 1c, 2c, 3c, 4, 5a and 6a were isolated for the first time from Pakistan (Idrees and Riazuddin, 2008). It has been confirmed by other studies that the most prevalent genotype in Pakistan is 3a with rate of 50% followed by genotype 3b and 1a, respectively (Idrees et al., 2008; Idrees and Riazuddin, 2008; Idrees, 2008; Sarwat et al., 2008).

Pathogenesis of HCV

Hepatitis C virus (HCV) causes acute and chronic heaptitis and approximately 85% of HCV infections progress to chronic infections, which often results in liver disease including variable degree of hepatic inflammation, oxidative stress, steatosis, fibrosis, cirrhosis, hepatocellular carcinoma and insulin resistance (Hoofnagle, 2002; Jacobs et al., 2005; Alter, 1997). In Pakistan approximately 80% of HCV cases develop to chronic infection which may increase in the coming decade (Idrees and Riazuddin, 2009; Khan et al., 2008). Hepatitis C virus RNA synthesis and protein expression affect cell homeostasis by modulation of a wide range of activities, including gene expression alteration, cell signaling, transcriptional modulation, transformation, apoptosis, steatosis, fibrosis, oxidative stress, membrane rearrangement, vesicular trafficking and immune response (Basu et al., 2006; Chang et al., 2008; Li et al., 2007). HCV is now viewed as a true metabolic syndrome associated with type II diabetes, hypertension, dyslipidemia, cardiovascular disease and atherosclerosis (Sheikh et al., 2008).

Mode of transmissions of HCV

HCV is blood born pathogen and high-risk have been observed in the recipients of multiple or repeated blood transfusions or blood products, intravenous drug abusers, prisoners, hemodialysis patients, healthcare workers exposed to needle stick and sharps injuries. In about 50% of infected patients (so-called 'sporadic' cases) have no obvious risk factor (Roman et al., 2008; Farhana et al., 2009).

In Pakistan approximately 70% of the cases were acquired in the hospitals via reuse of syringes and major/minor surgery that is very common in Pakistan (Idrees and Riazuddin, 2008). The overall observed mode of transmission in Pakistan were: multiple use of needles/syringes (61.45%), major/minor surgery/dental procedures (10.62%), blood transfusion and blood products (4.26%), sharing razors during shaving or circumcision by barbers (3.90%), piercing instruments, nail clippers, tooth brushes, siwaks, in less than 1% due to needle stick, from infected mother to baby and sexual transmission. For about 20.35% subjects the mode of transmission is unclear in this country (Farhana et al., 2009; Idrees et al., 2008; Idrees and Riazuddin, 2008) that is very dangerous situation. Injecting vitamins and antibiotics are very common in cities, towns and villages of Pakistan that play a major role in the HCV infection spread. Several studies from Pakistan showed that the average number of injections per person per year is more than 9 injunctions in this country that is 1 of the highest frequencies of injections anywhere in the world (Jafri et al. 2006; Khan et al. 2000). In addition, at a time of previous mass vaccination about 25 - 30 years back there was no concept of safe injection practices was largely received at schools and

villages that might be a source of HCV contamination. Other studies also reported that several times vaccinations at the public health-care facilities included sharing of syringes (Jafri et al., 2006; Khan et al., 2000; Ministry of Health, 2002). It has been established from Pakistan that subjects who had received more injections were more likely to be infected with HCV and these non-sterile syringes or needles may be the source of HCV infections (Idrees et al., 2008; Jafri et al., 2006; Khan et al., 2000). Presently the major source of hepatitis spread in this country is the use of previously used re-pack syringes. Even the Ministry of Health Government of Pakistan has published a survey in year 2002 that shows that in Pakistan more than 72% therapeutic injections and 50% immunization injections in public health-care facilities are unsafe and potentially dangerous for the spread of infections including HCV. Several other studies shows that even still the use of multiple-dose vials is common in many government and private sector hospitals that may also be an important risk factor in the transmission of HCV infection ((Jafri et al., 2006; Khan et al., 2000; Ministry of Health, 2002; Siddiqi et al., 2002). In many areas of Pakistan still sharing razors during shaving by barbers are common. Majority of the males, 20 years and older generally use barber shops for their shaving needs. According to a recent study higher anti-HCV prevalence in males compared to females could be due to this additional exposure to used and non-sterile shaving blades (Idrees et al., 2008). The study further describes that in 9% female subjects the probable transmission mode was the sharing of piercing needle/instrument. In females, piercing of nose and ears in group settings using non sterilized needles is a common practice in the area. According to this recent study, some of minor risk factors included sharing nail clippers, tooth brushes, Siwak, needle stick, from infected mother to baby and sexual transmission. The authors of that study have mentioned that in about ¼th of the anti-HCV positive subjects it could not ascertain any known risk factor and the transmission of infection is sporadic in this country.

Diagnosis and treatment

Diagnosis of hepatitis C is based on serological assays and HCV RNA. For screening and epidemiological surveillance enzyme-linked Immunosorbant assay (ELISA) and a confirmatory recombinant Immunoblot assay are initially used which detect HCV-specific antibodies (anti-HCV). Qualitative Polymerase chain reaction (PCR) is used to find out the presence of the viral genome in order to confirm active infection. Quantitative PCR is also helpful to monitor disease activity and response to treatment (Farhana et al., 2009).

Apart from quantitative PCR, genotyping has become increasingly important for routine laboratory diagnosis and the genotype should be taken into consideration when prescribing therapy. Both the duration and sustained response to current standard therapy regimens are strongly associated with the HCV genotypes (Idrees, 2008; Davis and Lau, 1997; Heathcote et al., 2000; Zeuzem et al., 2000). Evidence suggested that the patient with type 2 and type 3 HCV infections are more likely to have a sustained response to therapy than patient with type 1 (Idrees, 2008; Dusheiko et al., 1996).

In Pakistan, about 60 to 70% of the patients having a sustained virological response to therapy but at least 75% of patients have no therapeutic benefit (Mujeeb et al., 1997). In Pakistan more than 75% of Pakistanis are living below the line of poverty. Though more than 80 different brands of interferons are available in the market of this country, however they are very costly and are out of the reach of poor HCV infected patients. From 2006 - 2008, about 20,000 patients received interferon treatment free of charge from “Prime Minister Program for the prevention and Control of Hepatitis” but that is only 0.01% of the total cases and even after 12 years 3 out of 4 patients are still far away from treatment. Currently, the combination of interferon alpha and nucleoside analogue ribavirin is recommended for Pakistani patients with high reported sustained viral response rates (Idrees and Riazuddin, 2009; Farhana et al., 2009). Our previous observations showed that end of treatment response rates to IFN plus ribavirin therapy is very high (67%) in Pakistan. And significantly higher sustained virological response (SVR) rates were observed in Pashtoon (69.2%) as compared to, Punjabi (45.5%), Sindhi (45.5%) and Balochi (50%) for the patients received IFN-alpha plus ribavirin. In the same study we have seen highest SVR in patients with HCV genotype 2 (69.7%) followed by genotype 3 (57.3%) and lowest SVR in genotype 1 infection (24.3%) (Idrees and Riazuddin, 2009).

Recently, significantly increased response has been observed for polyethylenglycol (PEG)-conjugated interferon alpha as compared to conventional interferon alpha in the patients of genotype 1 (42 - 46%), genotypes 2 and 3 (76 - 82%) (Farhana et al., 2009; Foster and Mathurin, 2008). Side effects of interferon alpha are numerous and severe and require discontinuation of therapy in 2 - 10% of patients. The early side effects involve the inconvenience of subcutaneous administration of the medicine three times (or once) weekly for 6 - 12 months (Farhana et al., 2009; Foster and Mathurin, 2008).

Future need of studies

Currently no authentic country wide data is available on the actual prevalence rate of HCV in Pakistan (Idrees et al., 2008). In order to delineate the risk groups and risk factors of HCV infection it is important to conduct epidemiological studies that depict an accurate prevalence of the disease in the general population. A few epidemiological studies addressing the issue HCV prevalence

conducted in various parts of Pakistan used blood donors as the study population; it does not reflect an accurate prevalence of an infection in the general population of a country like Pakistan. Recently, Pakistan Medical research council (PMRC) has done a survey to find out the actual prevalence rate of hepatitis in Pakistan, which is important but the survey have low international credibility or none at all as the rapid test method was used for the screening of sera samples for the detection of anti-HCV. Here we suggest that the first and most important task is to find out the true prevalence of hepatitis C infection in the whole country at least using third generation ELISA for effective screening. In addition HCV RNA PCR should be performed for the anti-HCV positive sera to find out the true active HCV infection in Pakistan. In Pakistan about 90% of the HCV positive subjects are unaware of their HCV infection in general. So if we really want to defeat HCV, we must need to educate people and ensure that people know about that they are infected, so that they may receive anti-viral treatment and take steps to protect their colleagues, life partners, children and other family members. On government level Prime Minister of Islamic Republic of Pakistan had launched a program for the control and eradication of hepatitis from the whole country in year 2005. The program was very active in years 2006 - 2008 however; due to political changes in the country it is not functional as it was planned. Therefore it is suggested to resume the program and allocate more funds for at least 5 years. Additionally more efforts are also need on all levels to eradicate the problem from the country.

Several studies have already confirmed that genotyping is very important and useful test that should be done before starting anti-viral therapy (Idrees, 2008; Davis and Lau, 1997; Heathcote et al., 2000; Zeuzem et al., 2000). Therefore rapid, economic and reliable genotyping methods are required to be developed that may be used in routine in all clinical laboratories of the country. These locally developed assays will have more specificity and sensitivity as compared to other commercially available assays in the market.

Oxidative stress, fibrosis and steatosis generally seem to appear together in the liver with HCV infection and contributes in the development of Hepatocellular carcinoma (HCC) (Emerit et al., 2000; DeMaria et al., 1996; Rubbia-Brandt et al., 2000).In human HCV genotype 3, which is most common in Pakistan, is more commonly associated with steatosis (Idrees et al., 2008; Rubbia-Brandt et al., 2000; Roingeard and Hourioux, 2008). Moreover, association has been reported of genotype 3a core protein with lipid droplets (Rubbia-Brandt et al., 2000; Roingeard and Hourioux, 2008). Therefore the study of interaction between HCV protein and various genes involve in oxidative stress, fibrosis, steatosis and HCC development will very important and helpful for controlling HCV pathogenesis and effective drug designing for our local genotypes.

The current treatment is neither economical nor fully effective in all patients and carries significant side effects. Clearly, novel therapeutic strategies are urgently required in order to prevent the infection due to HCV. Several experimental anti HCV approaches have been presented recently and are discussed here at potential approaches to be implemented in Pakistan. Where therapy, is not widely available, vaccination is an advantageous option. Currently there is no approved anti HCV vaccine. It has been reported that infectious pseudo particle of HCV containing functional E1-E2 envelope protein complex mimic the early steps of parental HCV and may be suitable for the development of much needed antiviral therapies (Bartosch et al., 2003). Thus to prevent the infection due to HCV we will need to generate therapeutic vaccines by using pseudo particles of HCV 3a local genotype. This knowledge will also be helpful to study the early stages of viral life cycle like attachment and entry into host cells for our local setup. An additional experimental approach towards anti HCV vaccination could be nucleic acid immunization, which is the most recent approach in vaccine development. A DNA-based vaccine usually consists of purified plasmid DNA carrying sequences. Nucleic acid immunization is the most recent approach in vaccine development. A DNA-based vaccine usually consists of purified plasmid DNA carrying sequences encoding for an antigen of interest under the control of eukaryotic promoter. Many studies have been published on the development of DNA-based vaccines against HCV. It was reported that the injection into muscle cells of plasmids constructs expressing HCV core protein generate strong cytotoxic T-lymphocyte (CTL) activity, as assessed both *in vivo* and *in vitro*, and are promising candidates as antiviral agents (Tokushige et al., 1996; Gehring et al., 2004). The development of DNA base vaccine for our local setup will be valuable work and will be helpful in eradicating the whole nation from the blood born pathogen.

Finally, it has been suggested that RNA interference (RNAi) induced by small interfering RNA (siRNA), has potential as effective therapeutic agents for HCV infections (Trejo-Ávila et al., 2007). Synthetic siRNAs targeted against sequences in the protein-coding regions of core, NS3 and NS5B resulted in profound, up to 100-fold inhibition (Trejo-Ávila et al., 2007; Kapadia et al., 2003; Randall et al., 2003). siRNA is a rapid, inexpensive and sequence-specific gene silencing method. We suggest that such a study, concentrated on the 3a genotype which is common in Pakistan, will be helpful to catch potential therapeutic agents for our local genotypes and new therapeutic targets.

Conclusion

HCV infections represent a major threat to Pakistani population. There is a dreadful need to screen the whole nation for this “Silent Killer”. Presently no country wide

data is available about the actual prevalence rate of HCV in Pakistan. The development of diagnostic assays based on local genotype will be more sensitive, specific and economical. Due to the vital importance, HCV genotyping should be available in all clinical laboratories as a routine laboratory diagnosis and should be carried out before the start of anti-viral treatment. As the current treatment is neither economical nor fully effective in all patients and carries significant side effects, the need of the hour is to find out new therapeutic strategies and targets for local genotypes of HCV, using of siRNA technology and generation of protein and DNA base vaccines for our local setup.

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