ORIGINAL ARTICLE

Combination of Direct Antiviral Therapy in Hepatitis C Patients, Population of Karachi

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ABSTRACT

Background: Pakistan has approximately eight million Hepatitis C Virus (HCV) infected patients. Initial regimen of interferon-based along with ribavirin showed SVR (Sustained Virological Response Rate) of up to 50%. The new standard Direct Acting Antiviral (DAA) therapy with improved response rates raised SVR rates to as high as 90%. This study was conducted to determine the outcome of the novel combined DAA regimen in hepatitis C infected patients in Karachi, Pakistan.

Methods: Fifty patients with infected with HCV were participants of this study. They were from the gastroenterology ward and OPD Jinnah Medical Hospital (JMCH), Karachi. Initial investigations included blood samples for complete picture (CP) and liver function test (LFT). After performing qualitative Polymerase Chain Reaction (PCR), patients diagnosed with hepatitis C (pangenotypes) were prescribed DAA therapy.

Results: Out of total fifty patients diagnosed with HCV infection, forty compensated patients of hepatitis C were prescribed combination of Sofosbuvir and Velpatasvir, of these thirty five patients (100%) had shown to be PCR negative after three months of therapy and negative PCR after 3 months follow-up, five patients were lost to follow up. Ten patients decompensated (with ascites, cirrhosis or hepatic encephalopathy) were prescribed Sofosbuvir + Velpatasvir along with ribavirin, seven (100%) had shown to be PCR negative and three were lost to follow up.

Conclusion: Sofosbuvir and Velpatasvir was most effective combination of direct antiviral regimen in treatment of HCV pan-genotype patients, with least adverse-effects and much better outcome in both compensated and decompensated (with ascites and cirrhosis) hepatitis C infected patients.

Keywords: DAA-Direct-Acting Antiviral Agents; NS Protein; Polymerase Inhibitor; Protease Inhibitor; ETR-End Treatment Response, SVR-Sustained Virological Response.

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INTRODUCTION

HCV, an extremely serious and contagious infection has accounted for more than one hundred and seventy million patients worldwide. HCV is RNA virus, which is spread via blood and body fluids and is cause of morbidity[1,2]. Currently six HCV genotypes are identified as cause of worldwide infection, genotype 3a is the most prevalent HCV genotypes causing infection in 70% Pakistani population, followed by genotypes 1 is 7% and genotype 2 and 4 is 4%[3]. Latest direct antiviral drugs have shown potential for success due to their ability of inhibiting specific viral and host targets. The novel oral direct antiviral drugs have significantly increased the end therapeutic outcome for HCV infected cases; with SVR rates 90% reduced the therapeutic time duration[4].

Up to 2011, the established regimen for hepatitis C infection was pegylated interferon with or without ribavirin. After three months of this antiviral drug

therapy, the SVR rate was up to 50% only for genotype 1 patients and a response rate of 80% for other genotypes[3]. Interferon decreases the viral load by activation of the immune system (by JAK-STAT signaling to increase anti viral protein formation) and reduces viral load. Ribavirin is a nonspecific inhibitor of DNA and RNA viruses, by inhibiting guanosine triphosphate formation and blocks RNA polymerase and helps in viral clearance[4-6.] The interferon regimen had long duration from 24-48 weeks and has the disadvantage of unwanted adverse-effects and limited efficacy. Direct-acting antiviral agents (DAAs), a most effective HCV treatment, to eradicate spread of HCV and may reduce chronic liver disease by this infection and inhibit further spread[7]. Worldwide target given by World Health Organization is for eradicating spread of Hepatitis C by 2030[8,9]. The traditional interferon plus ribavirin therapy show SVR Sustained Virological Response (80%) for genotype 2 and 3 but interferon-based alone is 30-40% effective only[9,10].

The recent approval of oral direct antiviral agents (DAAs) for the treatment of HCV infection has shown better outcome in relation to SVR and only cause mild side effects. DAAs decrease viral replication by targeting specifically HCV non-structural (NS) proteins, which are most important for viral replication. First generation DAAs, Telaprevir and Boceprevir, act by inhibiting target site NS3/4A used with interferon and ribavirin for genotype 1 with improved SVR (70%) but might cause serious adverse effects. Whereas the second-generation NS3/4A protease inhibitors (Simpervir), NS5A inhibitors that inhibit protein hyperphosphorylation (Ledipasvir, Velpatasvir) and NS5B inhibitors (Sofosbuvir) have much better outcome and mild adverse effects. Direct antiviral therapy act by inhibiting specific viral targets site appear to have shown much better antiviral therapy outcome[9].

Combined direct antiviral agents are the most resent advancement for the treatment of HCV infection. Simeprevir / Sofosbuvir antiviral regimen has shown to be been highly beneficial in reducing viral load with mild side-effects[11]. A study on combination therapy of Sofosbuvir-Ledipasvir in combination with Ribavirin has reported to cure most patients with HCV, even in the presence of compensated liver cirrhosis[12]. A study had done on regimen of Mericitabine and Sofosbuvir both have shown highly beneficial results[10]. Thus, DAA combination regimens have shown very potent activity against all HCV infected patients, along with those not responding to traditional therapy[7-12].

Combination regimen of Simeprevir + Peginterferon-alfa 2a / 2b with or without ribavirin had shown much better reduction in viral load in treatment of patients with HCV genotype 1 infection, without causing serious adverse effects related with interferon–based regimen. The new regimen for HCV has shown dramatic improvement with the approval of two new oral DAA drugs used with or without pegylated interferon-based and ribavirin-based regimen. Studies have shown improved end treatment response rates and shortened duration of therapy for many patients with HCV infection[13,14]. NS3 viral protein with its Cofactor NS4 has protease activity essential for viral maturation, thus a great target for antiviral drug. In comparison to interferon therapy, the initial oral agents introduced were telaprevir and boceprevir (protease inhibitors) have shown much better outcome for hepatitis C virus (HCV) infection. Thus new regimen for HCV infection had greatly improved the outcome with the approval of these oral DAA drugs used with traditional interferon-based along with ribavirin-based triple therapy in 2011. Studies have shown improvement in response rates and shortened duration of therapy in genotype 1 HCV infected patients[15]. Chronic hepatitis C viral infection may cause serious complications, thus early detection and appropriate treatment is highly essential to prevent complications along with risk of hepatic cancer. The study was carried out to find out the outcome of combination of Sofosbuvir-Velpatasvir in hepatitis C infected patients of pangenotypes 1-6 in Karachi, Pakistan.

METHODS

Fifty HCV infected patients, diagnosed were included in this study from the gastroenterology ward and Medical OPD Jinnah Medical College, Hospital (JMCH), Karachi. Initial investigations including blood samples of patients were collected and biochemical investigation of complete blood count (CBC), LFT (Liver function test), Serum albumin, Prothrombin Time (PT), International Normalised Ratio (INR) and ultrasound abdomen were performed. The study approval was sort from the Jinnah Medical and Dental College, Ethics Review Committee.

After performing qualitative PCR (positive) were diagnosed of hepatitis C and prescribed antiviral therapy on diagnosis of all genotypes (Sofosbuvir /Velpatasvir400mg/ 100mg) once daily, for three months. End treatment results (ETR), PCR and ultrasound were repeated after three months of antiviral therapy. PCR was repeated after three months to obtain SVR. Total viral RNA was extracted from 150 µL serum samples by using Qiagen spin RNA virus extraction kit. The extraction was carried out according to the kit protocol instructions. Finally, each RNA sample was dissolved in 50 µL TE buffer.

HCV RNA was amplified by using real time amplification and detection kit HCV Real-TM Quant (Sansure, Biotech). Fluorescently labelled HCV specific probes were used. The reporter dye: FAM was used and Quencher: None was used for testing HCV-RNA. FAM dye was used to amplify HCV

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internal control (IC). Internal control was provided as an amplification control for each sample being processed to detect the possible contamination risks. Four standards were used from A to D having concentration (5.00E+06 IU/ml to 5.00E+03 IU/ml), Two positive controls for different quantities (quantities of standard 125 IU/mL and 12,500,000 IU/mL) and two negative controls were carried out using cDNA as substrate and spiked with mimic target in each run.

From the total RNA extracted, 10µL was used as template; PCR mix was taken 49 µL and RT-PCR enhancer 1 µL. Mixed and amplification was carried out by standard method pre-denaturation and enzyme activation was 95°C for 1 minute for single cycle. The reverse transcription; stage 1: 60°C for 30 minutes for 1 cycle , stage 2: 95°C for 1 minute (cDNA –pre denaturation) for 1 cycle. The stage 3: 95°C for 15 sec and 60°C for 30 sec for 45 repeats. PCR reaction was performed in Sansure (SLAN, China). Results were analyzed by using SLAN PCR software v2.0d. Viral load was calculated with the help of Cy3 and Fam Ct value (Cy3 STD/Fam STD × 510,000 = No. of HCV IU/mL). Further, qPCR was being performed in a one-step, in which assays combine reverse transcription and PCR in a single tube and buffer, using a reverse transcriptase along with a DNA polymerase.

RESULTS

Out of total fifty patients diagnosed with hepatitis C, after performing their liver function test (LFTs) and ultrasound liver done to determine echogenicity. All of these patients were prescribed direct antiviral therapy and outcome was assessed after ETR and SVR. Out of total fifty patients with hepatitis, forty were compensated after three months of combination therapy with Sofobuvir + Velpatasvir out of which thirty-five achieved SVR (%) 100 in 12 weeks was achieved but five patients were loss to follow. In addition, ten decompensated patients with complications such as ascites, fibrosis or cirrhosis was prescribed a combination of Sofobuvir + Velpatasvir and ribavirin out of these seven responded well to therapy with SVR 100%, three of these patients were loss to follow.

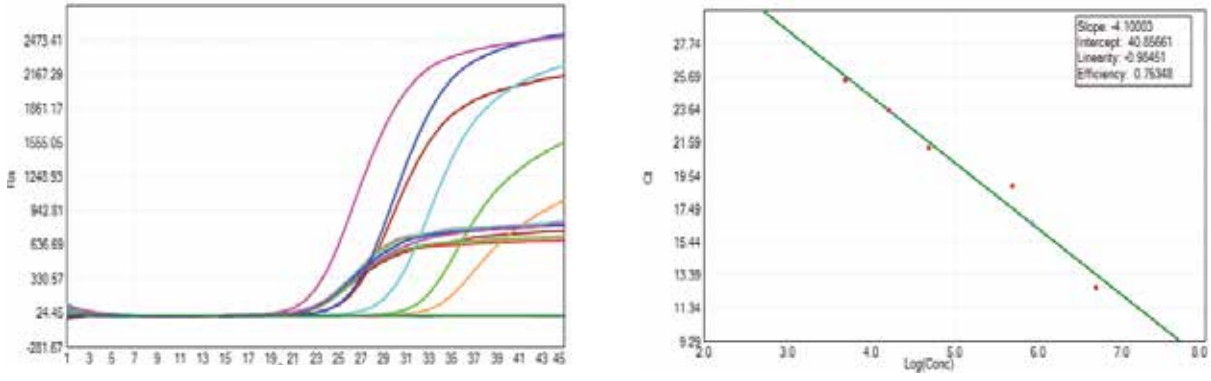
Figure 1: Hypoechogenic liver in hepatitis C patient.

Figure 1 shows hypoechoic hepatic parenchyma in-patient infected with hepatitis C. Combined Sofosbuvir + Velpatasvir therapy given in forty compensated hepatitis C patients for 3 months (12 weeks) shown to SVR 100% (Table1).

Table 1: Combined Sofosbuvir +Velpatasvir was efficacious and well tolerated in compensated hepatitis C patients.

No. of Patients N= 40	*qPCR before treatment	**DAA- therapy (3 months)	***ETR qPCR after three months treatment	****SVR (three months end of therapy)
n=35	Positive	Sofobuvir + Velpatasvir	Negative	Negative 35(100%)
n=05	Positive	Sofobuvir + Velpatasvir	Not Done (Lost to Follow up)	Not Done (Lost to Follow up)

**qPCR- qualitative PCR, **DAA- Direct antiviral therapy, ***ETR- End of Treatment, ****SVR Sustained Virological response.*



RNA vs. CYCLE **Standard Curve**

Figure 2: qPCR showing hepatitis C positive peak.

Ten decompensated patients (ascites, cirrhosis) were prescribed Combined Sofosbuvir + Velpatasvir along with ribavirin of these seven patients that followed the regimen for 3months shown to have SVR (100%) (Table 2). Figure 2 shows qPCR of hepatitis C positive peak.

Table 2: Sofosbuvir +Velpatasvir /Ribavirin was efficacious and well tolerated in decompensated hepatitis C patients (with ascites, cirrhosis).

No. of Patients N= 40	*qPCR before treatment	**DAA- therapy (3 months)	***ETR qPCR after three months treatment	****SVR qPCR after treatment
n=07	Positive	Sofobuvir + Velpatasvir + Ribavirin	Negative	Negative 7(100%)
n=03	Positive	Sofobuvir + Velpatasvir + Ribavirin	Not done (Lost to Follow up)	(Not Done) (Lost to Follow up)

qPCR- qualitative PCR, **DAA- Direct antiviral therapy,*ETR- End of Treatment, ****SVR Sustained Virological response.*

DISCUSSION

In Pakistan total population suffering from hepatitis is very high (second highest in world)[5-7]. Major issue is the lack of knowledge in these patients, infects with hepatitis C. Delay in early diagnoses and appropriate treatment results in development of chronic liver disease (ascites, fibrosis and hepatic cancer), and increases the financial burden on developing country such as Pakistan. Risk factors identified are, reuse of syringes and inappropriately screened blood for transfusion are common factors for spread of HCV, which vary from that of developed countries. Drug abuse by intravenous route of administration has been identified one of the major cause of spread of infection in developed countries. In Pakistan the reuse of needles/syringes was found to be the most alarming factor for spread of this highly contagious infections[6,7].

Sofosbuvir inhibits NS5B protein, of HCV. This drug is prescribed for infection caused by HCV genotypes 1 to 4, combined with other oral antiviral agents. Sofosbuvir inhibits RNA polymerase and is administered orally to treat HCV infection. Nucleotide analogues are activated by phosphorylation to nucleoside triphosphate in host hepatocyte, which competes with natural nucleotide, inhibiting RNA replication of the viral genome. The activated nucleotide triphosphate analogues act on highly active site of HCV (NS5B polymerase) causing chain termination[8-10]. Sofosbuvir alone, per oral at dose of 400 mg for one week had shown effectively reduce the viral load in patients with genotype 1 infection. Although, DAA therapy has a few upcoming challenges such as drug resistance, drug-drug interactions etc. Velpatasvir is NS5A protein inhibitors, blocks the action of protein and thus blocks HCV viral replication. Study done patients with chronic infection along with complications were prescribed combination DAA regimen.

New oral DAAs regimen include Sofosbuvir, and other oral RNA polymerase, are highly beneficial in providing interferon-free regimens for the treatment of HCV infection[14]. In study done in New Zealand from 2010-2011 on 40 patients hepatitis C randomization, all 10 patients SVR (100%) on Sofosbuvir plus ribavirin regimen without interferon, 30 patients (100% SVR), but only 6 of 10 patient with (60%) SVR who received Sofosbuvir monotherapy. Frequent side effects reported were headache, fatigue, insomnia, nausea and rash[15-18]. Previously the antiviral agent for hepatitis due to HCV infection was interferon-based therapy has parenteral administration thus caused frequent unwanted side-effects[19,20].

In our study, two groups of patients were prescribed combination of Sofobuvir + Velpatasvir only PCR (polymerase chain reaction) was done along with blood CP, LFT (SGPT and ALT raised), and serum albumin. After diagnosis on PCR (detectable HCV RNA), forty compensated patients were prescribed combination of Sofobuvir + Velpatasvir as direct antiviral therapy. And end therapeutic results indicator (ETR /SVR) of complete recovery in all thirty five patients had SVR (100%) patients after three months of antiviral therapy end of treatment (undetectable levels of HCV RNA) and follow-up sustained virological response, five patients were loss to follow. Ten decompensated patients (ascites, cirrhosis or encephalopathy) with hepatitis C infection were prescribed a combination of Sofosbuvir + Velpatasvir along with Ribavirin. All seven patients had SVR (100%) ETR/SVR seen (undetectable HCV RNA levels) after three months of antiviral therapy by repeating PCR. Three patients were lost to follow up.

Pakistan economy and health system are heavily burdened by hepatitis C infection (second highest in the world). The main issues are that these patients do not know they are infected or the extent of the liver diseases. A large pool of these patients is suffering from chronic infection causing complications such ascites, cirrhosis and hepatocellular cancer. The government has also made efforts such as, free diagnosis, treatment, and care for patients with HCV infection. According to current policies of

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government is to eradicate this infection. Patients infected with HCV will be provided free, oral antiviral medicines. These measures are taken by the Pakistani government show that commitment is necessary for eradication of hepatitis C and its associated morbidity and mortality[21]. In addition to high SVR, direct antiviral therapy is more cost effective with negligible adverse effects as compared to interferon-based therapy[21-27].

In WHO, it was approved that, most essential steps to eradicate the infection was improvements in detection and care, also by reducing the cost of oral direct antiviral drugs HCV infection. Screening for hepatitis C along with preventive interventions and appropriate therapy is mandatory. WHO guidelines were to follow injection safety, to ensure safe disposal of used syringes in health sector, to prevent reuse of syringes and eradication of major risk factor for spread of HCV in the country[27].

In the study on combination therapy had shown to be most active in reducing the SVR with least adverse effects for patients with hepatitis C virus genotypes 1to 6 even with complications. Such as advanced fibrosis or compensated cirrhosis. Sofosbuvir - Velpatasvir was reported to be highly effective in hepatitis C (genotype 1-6) infection also outcome was significantly beneficial in compensated patients with cirrhosis or advanced fibrosis. Forty-four per cent of these patients included in study had developed cirrhosis. SVR 12 weeks after treatment rates were 100% for hepatitis C virus genotypes 2, 4, 5, and 6. SVR 12 weeks after treatment rates was reported to be 98% in hepatitis C virus genotype 1 patients and 95% in hepatitis C virus genotype 3 patients. Among patients with cirrhosis 96%, SVR was achieved after treatment. Eight patients reported mild adverse effects. No patients discontinued treatment due to adverse events. Drug – drug interactions have been identified, administration of Velpatasvir/Sofosbuvir with the proton pump inhibitor (omeprazole) is not recommended, or should be taken 4 hours before DAA if necessary[28]. In addition, the concentration and activity of Velpatasvir is reduced with increase in gastric pH value. Prevention is the key to fighting against viral hepatitis spread.

LIMITATIONS OF STUDY

Sample size was small. Further studies with larger sample size and other combinations of direct antiviral therapy can be performed to analyze antiviral resistance.

CONCLUSION

Combination of oral direct antiviral therapy has shown much better outcome in both compensated and decompensated patients diagnosed with hepatitis C. No serious adverse effects were seen in this regimen of direct antiviral therapy. Thus, the new DAA therapy has lead to a major shift in HCV management, towards an IFN-free regimen. By adopting preventive measures (early detection, proper syringe disposal after use and screened blood transfusions) can eliminate/ reduce the spread of viral hepatitis.

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CONFLICT OF INTEREST

There was no conflict of interest among the authors.

ETHICS APPROVAL

The study approval was sort from the Jinnah Medical and Dental College, Ethics Review Committee.

PATIENT CONSENT

Verbal and written informed consent was obtained from all patients.

AUTHORS' CONTRIBUTIONS

SPK conceived the idea, wrote the manuscript, AK helped in sampling and bench work, MA and RG helped in sampling and helped in designing of the project, FAS facilitated in and data collection, SPK overall supervised the project and finalized the manuscript.

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