Original Article

A comparative study to evaluate effects on HCV-RNA-PCR by current oral anti-viral therapy with Sofosbuvir and Daclatasvir in Hepatitis C patients with and without Diabetes Mellitus in Pakistan

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Abstract

Objective: To compare the effects of antiviral therapy such as sofosbuvir and daclatasvir in HCV patients who were diagnosed by HCV-RNA PCR without Diabetes Mellitus.

Methodology: In this cross-sectional analysis total of 100 Hepatitis c infected patients selected, Sofosbuvir plus Daclatasvir treatment was given for 03 months period. Different parameters were recorded *such as* Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, Hb levels, Serum ALT levels, and Serum ALP level. The statistical relation of the mentioned variables was analyzed through SPSS version 15.

Results: Out of a total of 100 patients, 47% (47) were males, 53% (53) were females, 44% (44) patients had a history of prior interferon therapy, 23% (23) patients were having low hemoglobin levels before starting treatment. Both groups completed oral antiviral treatment for 12 weeks and resulting data showed the equality of treatment on group B and group A as no decrease in hemoglobin (p=0.799), ALT normalization (p=1.000), and no rise in serum bilirubin (p=0.817) during 1st month of treatment was noted in both groups while the SVR noted of both groups also showed no significant difference to each other i.e. 92% & 94% (p=0.696).

Conclusion: This study concluded that Sofosbuvir/ Daclatasvir tablets oral course of antiviral therapy against hepatitis C infected patients for 12 weeks showed excellent results, beneficial for the patients suffering from diabetes mellitus along with hepatitis C infection as compared with antiviral therapy for hepatitis C infected patients without diabetes

Keywords: Deviated nasal septum, nasal septum corrective surgery, sub-mucous resection, septoplasty.

Introduction

When we talk about liver cancer it is the 5th most common in males and 9th in females,[1] alarming situation enhanced the burden on healthcare institutions due to poor prognosis of liver-related cancer. When we talk about the mortality rating in the world it comes in 3rd leading cause of cancer mortality in 2013 reports[2]. In developed countries, the proportion of HCV infections leads to hepatic cellular carcinoma and primary liver cancer.[3,4]

The best strategies are applied to reduce the burden of hepatic cellular carcinoma to increased uptake of direct-acting antiviral treatment.[5,6] Reig et al. studied showed that that direct-acting antiviral treatment could increase the risk of hepatic cellular carcinoma recurrence[7] consider uncertainty direct-acting antiviral treatment and hepatic cellular carcinoma risk.[8,9] Previously meta-analysis estimated the level of risk reduction in patients cured versus non-responders was 77%.[10]

In the Pakistani population, the hepatitis load is approximately between 3-13% of the total population.[11] After the diagnosis of Hepatitis c infection, unfortunately, thinking about diabetes may not be 1st issue that comes to mind, both diseases are interlinked with each other is an unsolved issue. Their co-existence, unfortunately, can speed up the dangerous effects of hepatitis C and raised the chances of a serious liver injury-related disorder. Insulin resistance is known as a warning sign for Hepatitis C patients lead to diabetes mellitus disorder. In worldwide chronic hepatitis C virus (HCV) infection is very high, in 2013 reported population was about 170 million.[12] Decompensated liver cirrhosis together with hepatocellular carcinoma due to chronic HCV infection is the most common cause of death.[13] Nowadays, chronic HCV infection is considered a systemic disease because it does not affect only the liver but other organs. Diabetes mellitus is the most common extrahepatic manifestations of chronic HCV infection.[14]

Currently, oral treatment given by the combination of Sofosbuvir and Daclatasvir tablets is used to treat Hepatitis C infection. Sofosbuvir is the only known nucleoside analog that directly binds with the active site of polymerase/NS5B[15] of hepatitis C virus, so it is highly effective against all genotypes and possesses a high barrier to resistance. While Daclatasvir is an NS5A inhibitor[16] effective against all genotypes and possesses a low resistance barrier. So, adequate combination treatment prefers to overcome this resistance. The antiviral therapy response was measured by sensitive HCV-PCR testing having low detection limits up to $\leq$ 15 IU / ml in a patient.[17]

The primary goal of chronic HCV infection treatment is achieved after the sustained viral response (SVR), characterized by complete negativity of the hepatitis C virus from the patient's body. SVR is associated with decreased liver disease and mortality rate together with all-cause mortality rate.[18] Diabetic is defined if the patient has fasting plasma glucose levels equal to or more than 126 mg/dl in three consecutive results.[19] If PCR shows the negative result at treatment end known as End Treatment Response (ETR) while after 12 weeks of therapy completion known as Sustained Virological Response (SVR).[20] Internationally, SVR is known as the finishing point for anti-viral therapy against Hepatitis C patients.[21]

The HCV positive genome is composed of single-stranded RNA approximately 9600 nucleotides with a single open reading frame flanked by 5′- and 3′-untranslated regions. The 5′-UTR contains six secondary structure domains termed stem-loops (SLs) I–VI. SLII, SLIII, and SLIV form internal ribosome entry site that facilitates the translation of cap-less HCV RNA.[22]

The 5′-UTR contains essential replication signals for -ve RNA, 3′-UTR highly conserved and essential for HCV replication process.[23-25]

HCV belongs in the *Flaviviridae* family, enveloped virus encompasses single, + ve RNA stranded with 9.6 kb lengthy set of polyproteins up to 3,010 amino acids, [26] polyprotein precursors are co- or post translated processed by cellular and viral proteases to produce functional structural and non-structural proteins.[27] The structural proteins consist of core protein, C, and envelope glycoproteins E1 and E2, non-structural proteins included protease NS2, multifunctional protein NS3 having serine protease and helicase enzyme, serine protease cofactor NS4A, proteins NS4B and NS5A, and RNA-dependent RNA polymerase NS5B form complex which is responsible for HCV replication.[28]

Hepatitis C virus enters a susceptible host either directly, through needle inoculation or transfusion of contaminated blood products, or inadvertently through breakage of a percutaneous barrier (as exemplified by sexual or perinatal transmission) [29].virus enters in the hepatocytes or other susceptible cells through the viral receptor.[30] Total 3% world's population approximately 180 million affected with

HCV, Egypt (22%), Pakistan (4.8%), and china (3.2%) (31).

Materials and Methods

In this study different items used such as gloves, disposable syringes, forceps, spirit cotton swab, simple cotton swab, EDTA vials, scissor, Test tubes, tourniquet, Test tube stands, ultracentrifuge, Specimen containers, test tube holder, cuvettes, serological tubes (6.6*50 mm, kimbleno. 45060), pipettes tips (supply), micropipettes (Medilines) ρ-Nitrophenyl phosphate, ρ-nitrophenol, inorganic phosphate, P-NPP., oxoglutarate, L-Aspartate, GOT, glutamate, oxaloacetate, NADH, MDH, malate, NAD, heparin, ETDA, diazotized sulfanilic acid, sulfanilic acid, azo-bilirubin, drugs reagents, physiological saline(0.9%), sodium hydro-oxide and phosphate buffer.

PCR Machine (Thermocycler), laboratory centrifuge (Xinge Scientific Instruments Co.ltd), Coulter mixer (LH70), Albumin analyzer(Hi-Med), laboratory refrigerator freezer (HSE services), Spectrophotometer (Pyrocell), Environmental chamber (Zinis laboratories), Humidifier, Hematology analyser (Hi-Med), Vortex mixer (Lab mixer) (Analytical supplies) and Micro-plate centrifuge (Hi-Med).

It was a cross-sectional comparative research work done at Al-Rauf Medical & Surgical Hospital Sargodha Region from January to June 2018. In this study total, 100 patients were chronic hepatitis C disease positive, aged 18 years and above with positive HCV RNA selected. The exclusion criteria were patients with decompensated liver disease and child pug score >12, pregnancy, HIV or HBV co-infection, and renal dysfunction with Creatinine clearance <50 mL /minute. All patients have treated with Sofosbuvir and Daclatasvir combination for 12 weeks. Sofosbuvir's given dose was 400 mg daily, while Daclatasvir given dose was given 60mg daily according to the recommended dose and duration of oral antiviral therapy.

The selected people were divided into two groups containing 50 patients per group. 1st patients group included those patients which have Hepatitis C plus diabetes mellitus and named as group A while 2nd group included those patients which have Hepatitis C without diabetes mellitus and named as group B. Bio-data included patients age, sex, weight, prior interferon therapy, haemoglobin (Hb) level, Alanine aminotransferase (ALT) level, and bilirubin concentration noted. The changes in biochemical and haematological parameters were checked at 4 weeks of therapy. The serum HCV-RNA testing was performed at the end of 12 weeks and to see for ETR and SVR-12 respectively.

Tests were performed with a serum sample which is taken after centrifugation(7500rpm) for 15 min. The serum level of ALP was determined by the spectrophotometric method also using an alkaline phosphatase kit (Tietz.,1986). Serum bilirubin (total, direct and indirect bilirubin), and other parameters were measured by appropriate analytical methods using the spectrophotometer method for accurate results (Kaplan, 1984). Hemoglobin estimation was done by Sahil's method, Estimation of ALT was done by Fortress diagnostic kit, Estimation of Alkaline Phosphatase, and serum total bilirubin was done by Fortress diagnostic kit. The statistical relation of the mentioned variables was analyzed through SPSS version 15.

Results

There was a total of 100 patients with mean age 44.24 ± 11.20 years, weight range was 38 to 113 kg with a mean value of 73.35 + 13.00 kg. The mean values of baseline Hb, ALT, and bilirubin were 13.04 + 1.68 g/dl, 82.04 + 66.27 IU/ml, respectively (Table 1).

Table 1: Descriptive statistics of quantitative variables

Quantitative Variables	Min	Max	Mean ± SD
Age (Years)	19	75	44.24 ± 11.20
Weight (Kg)	38	113	73.35 ± 13.00
Baseline Hb (g/dl)	8.9	17.3	13.04 ± 1.68
Baseline ALT (IU/L)	12	623	82.02 ± 66.27
Baseline Bilirubin (mg/dl)	0.2	4.6	0.98 ± 0.66
Hb at week 4 of therapy (g/dl)	6.5	16	11.77 ± 1.79
ALT at week 4 of therapy (IU/L)	11	122	26.56 ± 15.58
Serum Bilirubin at week 4 of therapy (mg/dl)	0.1	5.5	0.92 ± 0.59

(n = 100)

Out of the total of 100 patients, 46% (46) were males and 54% (54) were females while 44% (44) patients have a history of prior interferon therapy, 23% (23) patients were anemic before starting antiviral therapy (Table 2).

Table 2: Qualitative variables (n = 100).

Variables /Categories	Group		Total
	Diabetic	Non-Diabetic	
Gender			
Male	23 (46%)	23 (46%)	46 (46%)
Female	27 (54%)	27 (54%)	54 (54%)
H/O Interferon therapy			
Yes	22 (44%)	22 (44%)	44 (44%)
No	28 (56%)	28 (56%)	56 (56%)
ALT normalization during the first month of therapy			
Yes	45 (90%)	45 (90%)	90 (90%)
No	5 (10%)	5 (10%)	10 (10%)
Baseline Hb			
Normal	38 (76%)	39 (78%)	77 (77%)
Low	12 (24%)	11 (22%)	23 (23%)

50 patients in each group A and B completed oral anti-hepatitis C therapy having and not having diabetes mellitus respectively. The ETR was achieved in 47 % in group A while in the B group it was noted 48% without showing any significant variation of results. The SVR also did not show any significant difference in results in group A & group B of the patients and was noted 93% in both groups A & B irrespective of being or not being diabetic. The equality of group A therapy with group B was observed in statistical figures. No decline in Hb> 2g/dl during the first month of therapy (p=0.799), ALT normalization during the first month of therapy (p=1.000), and no rise in serum bilirubin during the first month of therapy (p=0.817) were seen not significantly in both groups of patients. A decrease in Hb> 2g/dl during the first month of therapy was seen in only 9% of both groups and hence showed similar results to each other in terms of anemia. Similarly, ALT normalization during the first month of therapy was seen in 90% of individuals of both the groups of the patients. The serum bilirubin became elevated than normal value during the first month of therapy in only 7% of both groups of the patients, hence it proved insignificant (Table-3).

Table 3: Comparison of response to therapy as well as hematological & biochemical parameters (n = 100).

Predictors /Categories	Group		Total	p-value	OR with 95% CI
	Diabetic (n=50)	*Non-diabetic (n=50)*			
ETR					
Achieved	47 (94%)	48 (96%)	95	0.649	1.532 (0.249 - 9.587)
Not achieved	3 (6%)	2 (4%)	5		
SVR:					
Achieved	46 (92%)	47 (94%)	93	0.696	1.362 (0.289 - 6.426)
Not achieved	4 (8%)	3(6%)	7		
Decrease in Hb> 2g/dl during first month of therapy					
Yes	10 (20%)	9 (18%)	19	0.799	0.878 (0.323 - 2.388)
No	40 (80%)	41 (82%)	81		
ALT normalization during first month of therapy					
Yes	45 (90%)	45 (90%)	90	1.000	1.000 (0.271 - 3.694)
No	5 (10%)	5 (10%)	10		
Rise in Serum Bilirubin during first month of therapy					
Yes	4 (8%)	3 (6%)	7	0.817	1.112 (0.449 - 2.752)
No	46 (92%)	47 (94%)	93		

H/O = History of

Discussion

All trials done on oral antiviral therapy i.e. Sofosbuvir/ Daclatasvir against hepatitis C patients showed both ETR and SVR above 90%. Hence now the same combination is recommended in all HCV genotypes. In our study, ETR was achieved excellently in group A and group B of patients i.e. 94% and 96% respectively. Along with SVR which was also almost equally good with the said oral antiviral therapy in both diabetic as well as non-diabetic group individuals with very minimal difference (i.e. 92% in group A and 94% in group B patients). This suggests the same potency of oral antiviral therapy i.e. Sofosbuvir/ Daclatasvir therapy of 12 weeks duration in our

population and there was no significant difference between diabetic and non-diabetic hepatitis C infected patient groups (p-value=0.696 with 95% confidence interval).

Similarly, ALT normalization during the first month of therapy was seen the same in patients treated with Sofosbuvir/ Daclatasvir oral antiviral regimen in groups A and B (90% each). In our study, only two parameters were compared in two different groups of patients. The patients receiving oral treatment of both groups suffered a decrease in Hb> 2 g/dl during the first month of therapy though to a very minimal extent but almost equally i.e. 20% and 18% in group A and group B subjects respectively. Similarly, both A and B groups had an equal rise in serum bilirubin levels during the first month of therapy (8% versus 6%). Hence a very few patients suffered hemolytic anemia due to the said oral treatment of hepatitis C infected patients whether diabetic or non-diabetic. This whole scenario was suggestive that Sofosbuvir/ Daclatasvir therapy oral anti-viral treatment was equally efficacious for group A and B patients irrespective of having or not having diabetes mellites and had fewer side effects in our population in Pakistan.

Conclusion

This study concluded that Sofosbuvir/ Daclatasvir tablets oral course of antiviral therapy against hepatitis C infected patients for 12 weeks showed excellent results, beneficial for the patients suffering from diabetes mellitus along with hepatitis C infection as compared with antiviral therapy for hepatitis C infected patients without diabetes. The concept of co-existence of hepatitis c plus diabetes which told that individuals containing one of them are more prone and similarly vanishing HCV infection lowers the risks of diabetes; achievement of SVR lead to drop prevalence of Diabetes Mellites in patients with chronic HCV infection shortly. Insulin resistance leads to a worse therapeutic response against insulin therapy in patients with chronic HCV infection.

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