Pharmacokinetics profile of serum and cellular Sofosbuvir along with its concentration effect analysis in HCV patients receiving Sofosbuvir and Ribavirin

Bilal Ahmed[1], Bushra Munir[1], Abdul Ghaffar[1]*, Muhammad Yameen[1], Fatima Jalal[2], Muhammad Umar Farooq[1], Saba Shehzadi[1], Sara Ashraf[1] and Ayesha Liaqat[1]

[1]Department of Biochemistry, Government College University, Faisalabad, Pakistan
[2]Department of Zoology, Government College University Faisalabad, Pakistan

Abstract: Sofosbuvir along with ribavirin is being widely used for treatment of HCV in Pakistan but it may show delayed response and reoccurrence of disease in some cases. The aim of the study was to investigate pharmacokinetics and concentration effect analysis of sofosbuvir. HCV patients (n=100) received 400 mg sofosbuvir along with low dose or weight based ribavirin (400 mg). Nonlinear mixed effects modeling (NONMEM) and unpaired t-test were used for the association of concentrations and treatment outcomes. Average day 10 sofosbuvir metabolite BM 331007 concentration was higher in patients having haemoglobin nadir value <10 g/dl compared to the patients having heamoglobin nadir value >10 g/dl (5.34 versus 4.87 pmol/10^6 cells; p=0.03). The average concentration trends of GS331007 at day 10 was towards being higher in the patients achieved sustained virologic response (SVR) as compare to the patients relapsed (5.19 versus 4.86 pmol/10^6 cells; p=0.05). Sofosbuvir (GS331007) thresholds concentration (suggested at day 10 through receiver operating characteristic curve) was 5.4 pmol/10^6 cells for SVR (p=0.05) and haemoglobin nadir cells was 6.3 pmol/10^6 with sensitivity and specificity of ≥60%. Dosing simulations shows that 400 mg sofosbuvir twice daily produce day 10 concentration range of 5.4 to 6.7 pmol/10^6 cells. The range of therapeutic values was identified for HCV patients receiving sofosbuvir in combination with ribavirin for 24 weeks, suggesting a potential pharmaceutical basis for individualized therapeutic dosing.

Keywords: Hepatitis C virus (HCV), Sustained Virologic Response (SVR), GS 331007, sovaldi

INTRODUCTION

Direct acting antiviral (DAA) approaches have revolutionized the treatment plan of HCV and have many advantages over interferon therapy. Although several combinations of antivirals have shown promising results for attaining SVR, still there is a probability of relapse of disease. Sofosbuvir and ribavirin in combination are widely used in Pakistan now-a-days for eradication of HCV. Sofosbuvir is a NS5B polymerase inhibitor that efficiently bind genotypes 2a and 3a (Chang, 2016; Christoph, 2016). Pharmacokinetics of these drugs affect overall response and successful viral elimination of virus from patients. The local environmental conditions, living style, socioeconomic circumstances and food habits of population vary from those where the drugs are actually produced. This is collectively called "geonetics"; the effect of local conditions on drug metabolism in human body (Curry *et al*., 2015; Desnoyer *et al*., 2016). Similarly, the response of body towards these drugs not only varies in these conditions, but also differs in different patients (Omar *et al*., 2018). Despite the big improvement of SVR rates in this difficult-to-treat patient group, the success comes at the cost of additional side effects, like severe anemia, rash or dyspepsia and pruritus and strongly increased expenses (Gane *et al*., 2014). The problems with DAA therapy still exist as 1) resistance, 2) high cost of these drugs 3) time duration of therapy with reference to specific genotype of HCV (Kowdley *et al*., 2014; Kirby *et al*., 2015). This study covers the use of sofosbuvir with either low dose or weight based ribavirin in HCV patients and concentration effect on the outcomes of therapy in open labeled ITT analysis. The ratio of the SVR was 80% (24 out 30 patients). The concentration of GS 331007 greatly affects the probability of SVR as low concentration in the serum indicates the delayed SVR or non-responding to the therapy (Lawitz *et al*., 2014; Hoffmann *et al*., 2015). The effective therapeutic response for DAA needs proper validation system especially at Government level. There is dire need to define the pharmacokinetic (PK) parameters for sofosbuvir that will be helpful for the best dose adjustment and increased the chances of likelihood of attaining SVR and minimizing the risk of side effects (Omata *et al*., 2014; Leroy *et al*., 2016).

The significance of this study is to find out 1) serum and intracellular PK of sofosbuvir 2) define the association of concentration effect for serum and intracellular sofosbuvir 3) identification of target concentration range that will enhances the rate of attaining SVR and decreased the risk of side effects 4) determination of sofosbuvir dosing strategies which achieve desire PK exposure in HCV treatment.

*Corresponding author: e-mail: aghaffaruaf@yahoo.com

MATERIALS AND METHODS

Patients and study design

The patients selected (n=100) were provided with written informed consent for this single centered open labeled trial. Study protocol was approved by ethical review committee of Government College University Faisalabad (GCUF/ERC/4188). HCV patients infected with genotype 3a having baseline platelet count ≥50000 cells/µl and haemoglobin level male ≥12g/dl and female ≥11g/dl were selected. The cirrhosis stage of all the patients was in compensated range (Ahmed et al., 2014; Rower *et al*., 2015).

The first phase of study was open labeled trial including 25 patients treated with sofosbuvir 400 mg along with low dose ribavirin (400 mg). Second phase of 75 patients were treated with sofosbuvir along with weight based ribavirin (<75 kg, 400 mg in day and 600 mg at night >75 kg 600 mg twice a day). Sofosbuvir and GS331007 were quantified from serum and RCB samples at 1, 2, 4, 8, 12 and 24 h.

Bioanalytical method

Sofosbuvir concentration in serum was determined by using a validated HPLC-MS method that was linear between range of 0.05 to 10 mg/l. The whole blood samples were drawn into PAXgene RNA isolation tubes (Nespak reagents, Lahore, Pakistan), and diluted 1:50 with 1 ml of 70% methanol solution before the extraction of RNA and injected to HPLC for identification and quantification. Purified red blood cells (RBCs) samples were used to establish strong correlation of concentration results (R^2=0.998). Water QMA anion exchanger with solid phase extraction (SPE) cartridge and dephosphosphorylated kit was used for the separation of sofosbuvir and its metabolite GS331007. The extraction essay was validated for linearity from 0.05 to 200 pmol/sample and concentrations were normalized to a per million cell count pmol/10^6 cells (Zeuzem *et al*., 2014). PAXgene (Pre analytics, BD, Japan) blood DNA kit was used for the genotyping essay of extracted DNA samples from the whole blood. TaqMan SNP genotyping essay was used for the PCR analysis.

Pharmacokinetics model

Characterization of sofosbuvir and GS331007 was done by using population PK modeling. Non-linear mix effect modeling essay (NONMEM version 7.2 new Hanover, USA) was utilized for the construction of population PK modeling. First ordered conditional estimation and additive and proportional errors were used for all models. Serum concentration of 256 samples from 100 patients were analyzed by using two compartment modeling through parameters clearance (Cl), absorbance rate (Ka), and elimination rate (Ke), intracellular formation (kin) and volume (V) act as scaler (Ke indicates association between covariance). The effect of sex, age, weight, serum creatinine and glomerular filtration rate (GFR) was calculated by using modification in diet MDRD equation.

Pharmacokinetics PK/Pharmacodynamics (PD) association

Average concentration (C_{ave}) from day 1-10 was determined by using final population PK model for sofosbuvir and GS331007. PD parameters were determined by using treatment outcomes like SVR or relapse, baseline hemoglobin (g/dl) hemoglobin nadir, change in hemoglobin, dose requirement for prevention of anemia. PK and PD association was done by using Pearson's rho correlation (continuous and unpaired t-test). Covariance was evaluated by using logistic regression analysis (SAS version 9.4). Finally receiving operator characters (ROC) curves were constructed for determination of threshold potential exposure.

Dosing simulations

Threshold concentrations were identified by using PK and PD correlation analysis to find out dose regimen. The SIM programmed in ADAPT was used to simulate the dose of sofosbuvir for quality or results. Ribavirin dose exposure daily was analyzed with different combination of ribavirin ranging 400 to 800 mg.

STATISTICAL ANALYSIS

Fibrosis stage (histology activity index (0-4) was confirmed and phenotyping on serum sofosbuvir and GS331007 was explored by using Pearson rho correlation for continue covariant and unpaired t-test. Graph-pad software was used for the covariant analysis by using cut off value $p<0.10$. Nested fashion was adopted for association between clinical factors and PK parameters. Significance value of $p<0.05$ was adopted throughout the modeling and covariant were added into forward inclusion fashion until no significant covariant was left.

RESULTS

Demographics of patients

Most of the patients had HCV genotype 3a (table 1). Minimal fibrosis (with mild to moderate) ratio was 73% based on the liver histology obtained by biopsy, average body weight was 78.5% and baseline HCV RNA level was ≤750000 IU/ml. The successful treatment was found in 88% patients to achieve SVR and 10 patients relapsed while 2 patients were dropped out from this study.

Population PK model

The median range serum concentration of sofosbuvir was 1.87 (0.85-5.56) mg/l and GS33100 was 9.37(3.43-13.37) mg/l (table 2). The half-life of sofosbuvir was 1.6 (1.4-1.9) days and GS33100 was 3.7 (2.9-5.6) days. The central compartmental volume (Vc) and estimator GFR

on CL parameters were the clinical covariates for sofosbuvir serum concentration. Higher level of Vc and GFR associated with frequent clearance of sofosbuvir almost 25% increased GFR represent 10% increased CL of sofosbuvir and its metabolite GS331007 (table 3).

Table 1: Demographics of HCV patients (n=100)

Gender	65 (65%) Males 35 (35%) Females
Weight (kg) median range	78.5 (58.5-122.34) kg
Age (Years) median range	54 (25-78) years
Baseline HCV RNA level	Males 65 >750000 IU/ml Females 35 ≤750000 IU/ml
HCV genotype	88 (88%) 3a 12 (12%) 1a
Fibrosis stage	23 (23%)= 0 48 (48%) =1 2 (2 %) =2 23 (23%)= 3 4 (4%) =4

This data was presented as mean value of n=100 participants' and AUC was 0-24 h for both the low dose and high dose groups. The low dose group shows promising results at the end so 400 mg sofosbuvir is enough concentration that best control on the progression of disease.

Biochemical parameters

The average levels of biochemical parameters for liver function test indicated cytolysis (table 4).

PK and PD association

The population means (%CV) was used as the associated values between PK and PD. The C_{ave} of sofosbuvir was ($p<0.002$ through day 10) while C_{ave} of GS 331007 was ($p<0.5$ on day 10). The value of sofosbuvir was inversely proportional to Hb nadir as results revealed that mean (SD) at day 10 of sofosbuvir was higher (6.68 (1.60) pmol /10^6cells) in those patients where Hb nadir was <10g/dl relative to those where Hb nadir ≥10g/dl (4.43(1.39) pmol 10^6 cells p=0.02). So it indicates that there is a need of readjustment in dose according to the level of Hb in patients' blood. The concentration of GS331007 was higher in those patients that achieved SVR the mean score was (4.64(1.56) pmol/10^6 cells) while the patients that relapsed or delayed SVR have mean concentration (4.09 (1.42) pmol/10^6cells: p=0.06). Baseline HCV RNA level ≤800000 IU/ml and female sex also associated with SVR. The odds SVR was 5.6 times greater (p=0.03) in females then males and the SVR was also achieved in those having HCV RNA level >800000 IU/ml. The multivariable logestic regression model was applied for both the variables and results showed statically significant relationship between HCV RNA level and achievement of SVR at day 10 of the study shown in fig. 1.

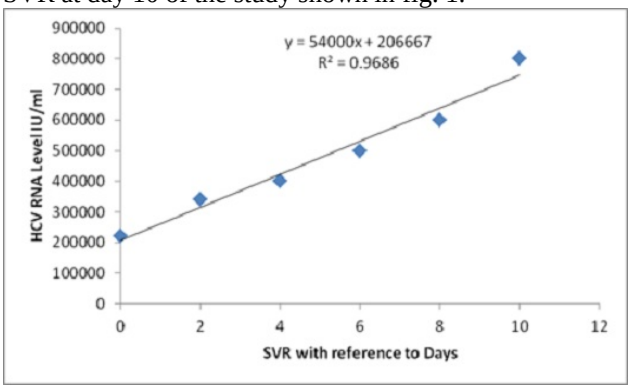

Fig. 1: Achievement of SVR with reference to HCV RNA level.

ROC analysis

The association between SVR and hemoglobin nadir with day 10 C_{ave} helps to identify exposure threshold using ROC curves (fig 2). The ROC curve of sofosbuvir at day 10 suggest a C_{ave} 4.1 pmol/10^6 cells for attaining SVR and for GS331007 C_{ave} 6.1 pmol/10^6 cells was necessary for attaining SVR. The SVR ROC curve had a AUC of 0.65 (p=0.05) while the curve described the Hb nadir AUC 0.81(p=0.02). The sensitive (true positive rate) of thresholds were 68% and 60% for Hb and anemia while the specificities true negative rate of thresholds were 75% and 82%. This suggest that treatment failure may occur in 75% patients when sofosbuvir concentration is <4.1 pmol/10^6 cells.

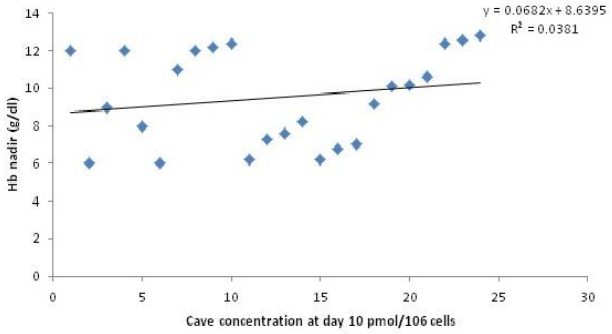

Fig. 2: C_{ave} concentration and nadir Hb values.

Dosing simulations

The dose of sofosbuvir and ribavirin was optimized and simulated for best of its results. The ribavirin dosing regimens that best fit for the attaining of SVR at day 10 and concentration values of sofosbuvir would be in therapeutic window of 4.1-6.1 pmol/10^6cells was optimized. The simulating average (SD) concentrations resulting from the various regimens presented in fig 3, where dose of 200 to 1400 mg/day was simulated and optimized concentration was assessed. The results indicate that 400 mg tablet will provide desire concentration for attaining SVR.

Table 2: Serum model parameters estimator of sofosbuvir

Parameters	Base model	Final model
CL (L/h)	16.6 (14.4-18.8) 35.3%	11.2+6.18=18.0 35.2%
V2 (L)	4410 (4210-46.10) 73%	Female 3311 34% Male 3840 48%
Q (L/h)	6.78 (1.14-12.78) 37%	6.97 (0.67-13.3) 43%
V3 (L)	2760 (1660-3860) 64%	2660 (1640-3680) 60%
k_α (h^{-1}) fixed	2.0	2.0

Mean data presented with confidence interval 95% having %age of inter individual variability.

Table 3: Steady state PK parameter estimator

Parameter of study	400 mg dose sofosbuvir	600 mg sofosbuvir
Serum concentration		
AUC (mg.h/l)	36.6 (38%)	39.04 (42%)
C_{ss} (mg/l)	1.38 (33%)	1.46 (36%)
$T_{1/2}$ days	2.4 (62%)	2.6 (64%)
Sofosbuvir parent drug		
AUC (pmol.h/10^6 cells)	172 (49%)	177 (51%)
C_{ss} (pmol/10^6 cells)	7.24 (47%)	7.48 (53%)
$T_{1/2}$ (days)	12.13 (22%)	11.36 (20%)
GS331007		
AUC (pmol.h/10^6 cells)	1234 (57%)	1366 (59%)
C_{ss} (pmol/10^6 cells)	112 (53%)	116 (54%)
$T_{1/2}$ (days)	11.56 (63%)	12.6 (62%)

Table 4: Average data of different biochemical parameters of HCV infected patients treated with direct acting antivirals

Biochemical parameters	Group- I Mean ± SD	Group -II Mean ± SD	Average
Albumin g/dl	4.26 ± 0.45	4.56 ± 0.13	4.58 ± 0.57
AST Folds IU/L	93.21 ± 1.37	91.92 ± 1.10	92.45 ± 1.38
ALT Folds IU/L	75.69 ± 2.17	74.91 ± 2.09	74.98 ± 2.11
GGT IU/L	98.61 ± 1.23	98.91 ± 1.11	98.64 ± 1.23
ALP folds IU/L	85.50 ± 2.13	84.81 ± 2.22	84.93 ± 2.15

Table 5: Average pharmacokinetics of sofosbuvir and its metabolite GS 331007

Parameters (units)	Average data sofosbuvir (N=100)	Average data of GS 3310077 (N=100)	Average data of ribavirin (N=100)
C_{max} (ng/ml)	746±261	1676±361	792±92
T_{max}(h)	1.30±0.61	3.30±0.91	1.8±0.2
AUC (ng.h/ml)	1036±212	16300±401	13100±103
$t_{1/2}$ (h)	2.4±0.92	9.80±2.7	21.4±3.2

C_{max} (ng/ml) Maximum concentration, T_{max}(h) Time for maximum concentration, AUC (ng.h/ml) Area under curve
$t_{1/2}$ (h) Time for half life

Pharmacokinetics parameters

The average C_{max} of sofosbuvir was 746 (261) ng/ml, GS331007 was 1676 (361) ng/ml and ribavirin was 792 (92) ng/ml. The average T_{max} for sofosbuvir was 1.30 (0.61) h, GS331007 was 3.30 (0.91) h and ribavirin was 1.8 (0.2) h. The average AUC (ng.h/ml) was 1036 (212),

GS331007 was 16300 (401) and ribavirin was 13100 (103). The $t_{1/2}$ for sofosbuvir was 2.4 (0.92) h, GS331007 was 9.80 (2.7) h and ribavirin was 21.4 (3.2) h.

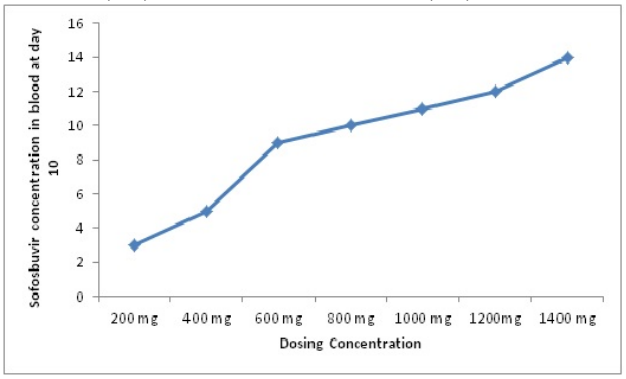

Fig. 3: Dosing simulations for optimum concentration of sofosbuvir for viral therapy.

DISCUSSION

This study was conducted for detailed analysis about the dose of sofosbuvir that best fits for the attaining of SVR within specified time duration of therapy. The study utilizes clinical samples to understand the serum and cellular disposition of sofosbuvir and its metabolite GS331007 and explore the PK/PD association of sofosbuvir and requirement of dosing for attaining SVR. Sofosbuvir half-life was found 2.4 h in serum and 4.6 in RBCs. The analysis between RNA level and attaining of SVR indicates that RNA level less the 800000 IU/ml was in favor of attaining SVR while increased value of RNA was very unfavorable for SVR. The Hb indicates that <10 g/dl Hb was unfavorable for attaining SVR while more than this nadir value shows significant improvement in results. Almost 90% patients of acute HCV achieved SVR with a mono-therapy of DAA, while in case of chronic HCV, even with combination therapy of sofosbuvir and ribavirin SVR range is only up-to 95%. Reasons for this discrepancy are unknown. The liver reaction of host of CHC patients was studied and established association of combination therapy and non-response to treatment, none of this has so far been analyzed in the HCV patients. Our results indicates positive predictors for SVR. Age under 50 years have more effective response of DAA therapy and rapid viral clearance rate while age above 50 have more complications although SVR was achieved but still there was severe effects of therapy even life threatened as well. Mild to moderate liver fibrosis have its impacts on SVR. The patients having mild liver fibrosis achieved 100% SVR with short 12 weeks therapy while the patients having moderate and advance liver fibrosis have some complications and SVR rate was low as compared to mild conditions.

The increased level of AST, ALT and GGT before the treatment was a negative predictor of SVR. DAA have effective role for virus clearance in short period of time while metabolic factors influence the efficacy of DAA specially biochemical parameters of the patients. Most of the patients attained SVR with DAA therapy patients with abnormalities in biochemical profiles showed some complications. The result of renal function test indicates its minor role for attaining SVR which depends upon the concentration of urea and creatinine in the urine of the patients. The normal ranges of RFTs are positive predictor for SVR with DAA.

Model estimates of sofosbuvir serum CL (CL/F=18.3 L/h) individual with median range renal function was similar to previous published reports, which ranged from 8.9 to 23.32 L/h (Afdhal *et al*., 2014). The sofosbuvir clearance was significantly correlated with GFR and relapse was more common in males than females. The model estimated serum half-life and estimated values were according to previous reports (Munir *et al*., 2017).

CONCLUSION

This is the first *in-vivo* study for the description of bioanalytical and pharmacokinetics parameters of direct acting antiviral sofosbuvir that is best suitable drug for eradication of HCV genotype 3a today in Pakistan. So for no suitable drug validation studies have been reported in Pakistan that best describe the possible dosing simulations according to the individualized conditions. The present study simulated dosing concentrations and find out the best fit value for attaining the SVR in short duration and for minimum side effects. The findings this study demonstrate that 400 mg drug secretes enough concentration in blood and RBCs that will be helpful for the complete eradication of HCV RNA from the body.

ACKNOWLEDGEMENT

The authors acknowledge higher education commission of Pakistan for funding this research under Grant No. 5568/Punjab/NRPU/R&D/HEC/2016.

REFERENCES

Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M and Foster GR (2014). Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *New Engl. J. Med.*, **370**: 1889-1898.

Ahmed B, Iqbal T, Mahboob H, Latif F and Sohail I (2014). Urinary excretion and renal clearance of nimesulide in male volunteers. *Glob. J. Med. Res.*, **14**: 804-811.

Chang ML (2016). Metabolic alterations and hepatitis C: From bench to bedside. *W. J. Gastroentero.*, **22**: 1461-1476.

Christoph S (2016). The importance of resistance to direct antiviral drugs in HCV infection in clinical practice. *J. Hepatol.*, **64**: 486-504.

Curry MP, Forns X, Chung R, Terrault NA, Brown JR and Fenkel JM (2015). Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology*, **148**: 100-107.

Desnoyer A, Pospai D, Le MP, Gervais A, Heurgue-Berlot A and Fontaine H (2016). Pharmacokinetics, safety and efficacy of a full dose sofosbuvir-based regimen given daily in hemodialysis patients with chronic hepatitis C. *J. Hepatol.*, **65**: 40-47.

Gane EJ, Stedman CA, Hyland RH, Ding X, Svarovskaia E and Symonds WT (2014). Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *New Engl. J. Med.*, **368**: 34-44.

Hoffmann FS, Schmidt A, Dittmann C, Wisskirchen M, Hellmuth J, Willms S, Gilmore H and Glas J (2015). Polymorphisms in melanoma differentiation-associated gene 5 link protein function to clearance of hepatitis C virus. *J. Hepatol.*, **60**: 460-470.

Kirby BJ, Symonds WT, Kearney BP and Mathias AA (2015). Pharmacokinetic, pharmacodynamic and drug-interaction profile of the hepatitis C virus NS5B polymerase inhibitor sofosbuvir. *Clin. Pharmacokinet.*, **54**: 677-690.

Kowdley KV, Gordon SC, Reddy KR, Rossaro L and Bernstein DE (2014). Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *New Engl. J. Med.*, **370**: 1879-1888.

Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M and Younoss ZM (2014). Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: The COSMOS randomized study. *The Lancet*, **384**: 1756-1765.

Leroy V, Angus P, Bronowicki JP, Dore GJ, Hezode C and Pianko S (2016). Daclatasvir, sofosbuvir and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+). *Hepatology*, **63**: 1430-1441.

Munir B, Ahmed B, Kiran S, Jalal F, Zahoor MK, Shehzadi S and Ghaffar A (2017). Sorafenib tosylate, ribavirn and ofosbuvir combination therapy for HCV virus infected patients with decompensated liver cancer. *Pak. J. Pharm. Sci*, **30**: 2383-2387.

Omar H, El Akel W, Elbaz T, El Kassas M, Elsaeed K, El Shazly H and AbdAllah M (2018). Generic daclatasvir plus sofosbuvir, with or without ribavirin, in treatment of chronic hepatitis C: Real-world results from 18 378 patients in Egypt. *Aliment. Pharm. Ther.*, **47**: 421-431.

Omata M, Nishiguchi S, Ueno Y, Mochizuki H, Izumi N and Ikeda F (2014). Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: An open label, phase 3 trial. *J. Viral Hepatitis*, **21**: 762-768.

Rower JE, Meissner EG, Jimmerson LC, Osinusi A, Sims Z and Petersen T (2015). Serum and cellular ribavirin pharmacokinetic and concentration effect analysis in HCV patients receiving sofosbuvir plus ribavirin. *J. Antimicrob. Chemoth*, **70**: 2322-2329.

Zeuzem S, Dusheiko GM, Salupere R, Mangia A, Flisiak R and Hyland RH (2014). Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *New Engl. J. Med.*, **370**: 1993-2001.