Evaluation of Direct-Acting Antiviral Drugs for Hepatitis C Genotype 3 Patients from ages 30-50 years in Sialkot, Pakistan

Kashif Waqas[1], Ayesha Saddiqa[2], Syed Zeeshan Haider Naqvi[1*], Javed Anver Qureshi[1], Omair Arshad Dar[3], Mazia Shahid Butt[4], Ishfaq Ahmad[1],Faheem Hadi[5], Tahir Maqbool[1*],Sana Javaid Awan[6]

[1]Centre of Research in Molecular Medicine, Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.
[2]Independent Medical College, University of Health Sciences, Lahore, Pakistan.
[3]Pak Emirates Military Hospital, Rawalpindi, Pakistan.
[4]Lahore General Hospital, Lahore, Pakistan.
[5]Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.
[6]Department of Zoology, Kinnaird College for Women, Lahore, Pakistan.

Correspondence to Dr. Syed Zeeshan Haider Naqvi
Centre of Research in Molecular Medicine, Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan, zeeshan.haider@imbb.uol.edu.pk

ABSTRACT

Background:

Pakistan is in the top ten countries which are affected by chronic Hepatitis C virus infection. Elimination of infection is now a possibility due to advancements in medical science in the current era. Hepatitis C is occurring with multiple genotypes such as 1, 2, 3, 4, and 5. Genotype 3 is most common in Asian countries as compared to other genotypes. Direct-acting antiviral drugs had replaced liver transplantation in many cases and little knowledge is available regarding follow-up studies on these drugs.

Methods: A follow-up research was performed to evaluate 3 months of treatment outcome of some direct-acting antiviral drugs in 228 patients (177 patients of HCV genotype 3) ages 30-50 years using some relevant biological parameters.

Results: A total of 208 patients yielded a pooled SVR of more than 90% after twelve weeks of outcome of commonly used antiviral drug evaluation.

Conclusion: Commonly used antiviral drugs in Sialkot, Pakistan showed effectiveness in HCV patients having genotypes 1, 2, 3, 4 & 5.

Keywords: Hepatitis C virus, genotype 3, direct-acting antiviral drugs, liver function tests, renal function tests.

INTRODUCTION

HCV infection is a prevailing disease that infected millions of people worldwide with an undetected prognosis in most cases and several communities are trying to eradicate HCV by 2030. Finding patients of HCV in the first move and providing them with cost-effective treatment in the second move is the order to eradication. An increase in age further increases treatment risk in HCV prevalence. This infection exists in multiple genotypes (1-6). Most of the patient suffer from genotype (1-3) and rarely from genotype (4-6). Most countries are affected by genotype 1 of HCV. Genotype (3) is more common in regions of South Asia and genotype 4 in Middle East countries. HCV genotype 3 patients are more likely to suffer from fibrosis, steatosis, and liver cancer in comparison with other genotypes (1). With the progression of infection, the fibrotic condition of the liver changes to cirrhosis, with the passage of time and even to intrahepatic cholangiocarcinoma and hepatocellular carcinoma in chronic stages which result in an increased rate of morbidity and mortality (2). Severe fibrosis of the liver may occur due to some risk factors such as age, gender, obesity, hepatic steatosis, and duration of HCV infection (3). Lack of basic safe and healthy water facilities, prevailing socioeconomic conditions, political statuses, and unstable healthcare delivery system results in increased chances of progression of infections including Hepatitis C in Pakistan (4,5).

Direct-acting anti-viral drugs had replaced conventional treatment for hepatitis C infection. These drugs displayed effective treatment of infection in different areas of the world, especially in combination which is also recommended by the European Association for Study of Liver treatment guidelines. These drugs combinations include grazoprevir plus elbasvir, glecaprevir plus pibrentasvir, sofosbuvir plus velpatasvir, sofosbuvir plus daclatasvir, ombitasvir/sofosbuvir/ledipasvir/ritonavir/paritaprevir plus dasabuvir). They are very convenient and displayed high SVR rates. But still, medical science is lacking real-world data regarding the outcome and follow-up of these drugs in cases of hepatitis C patients. Avoiding drug-drug interactions, especially in the case of antiretrovirals and direct-acting anti-virals, are also under discussion and another problem is that liver infection treatment is difficult to treat in advanced stages even by direct-acting anti-viral drugs but luckily infection can be minimized up to 75% through effective management. Consequently, treatment of HCV infection is included in the list of primary healthcare programs. Patients having other fatal diseases in combination with HCV infections such as thalassemia, HIV, and renal problems, is a major challenge in the treatment of HCVz

MATERIALSANDMETHODS

Materials: Specimen (patient's plasma), Gloves (latex or nitrile), blood collection tubes, pipette, centrifuge, micropipettes to dispense volumes 1-1000µl, with compatible sterile filtered tips, Roche® COBOS e411 auto analyzer for ELISA, Sysmex® KX-21 / Mindray® BC5000 automated hematology analyzers, Roche® COBAS c311 auto analyzer for routine chemistry, Roche® COBOS e411 auto analyzer for ECLIA special/hormonal assays, Roche® AMPLIPREP for automated Nucleic acids extraction), in association with COBAS TaqMan® and the CEPHID Smart Cycler by Thermofisher®.

Sampling: These include the patient's serum, plasma, EDTA, and citrated whole blood. All the samples were subjected to the relevant diagnostic workup. Peripheral blood was collected from each participant and serum/plasma was stored at - 800C for molecular assays (1).

Follow-up: Patients started on treatment with direct-acting antiviral drugs were reviewed after an evaluation based on the clinical, hematological, biochemical, and molecular assays depending upon their individual criteria and were followed up after 3 months of treatment.

Laboratory Methods:

Hematology: CBC on Sysmex® KX-21 or Mindray® BC5000 automated analyzers.

Clinical Chemistry: Routine chemistry tests like LFTs, RFTs, etc. were performed with Roche® COBAS c311 auto analyzer for routine chemistry. These parameters play an important role in detecting liver damage and injury (2).

Direct-Acting Antiviral Drugs: Current direct-acting antiviral drugs available in the local area are mentioned in table 1.

Inclusion criteria: Patients reporting to the outpatient department of Pak Medical Centre, Sialkot were interviewed and examined by the medical officers offering registration to the research enrolment. Presumptive Hepatitis C-positive cases of ages 30-50 years, identified by using the standardized WHO/Hepatitis Control Program (HCP) clinical diagnostic algorithms were enrolled. Consent in writing was obtained from all the participants. Patients having reactive HCV on ELISA with an age range of 07 – 29 years were enrolled in this study. Participants with high ALT levels (1.5 times more than the normal range) with a difference of 6 months and patients with co-morbidities like well-controlled diabetes and hypertension were included in this study.

Exclusion criteria: Not agreeing to participate in research work at any stage of treatment. Patients having platelets count less than 50,000/cubic mm. Patients with moderate to severe hepatic or renal insufficiency. Patients are co-infected with HBV. Pregnant females were not enrolled in this study. Patients having either extrahepatic malignancy or hepatocellular carcinoma.

Statistical analysis: Statistical analysis was performed with Graph Pad software, and all data of groups were expressed as mean ± SEM. For statistical analysis, groups were compared by unpaired t-test (two-tailed) with a 95% confidence interval. $P \leq 0.05$ was a threshold for statistical significance.

RESULTS

Available DirectAnti-Viral Drugs in Local Area

Locally available direct acting anti-viral drugs have been mentioned in table 1 with their administration on patients (ages and genotypes mentioned).

Table1: Currenttreatmentoptionsforgenotypes1,2,3, 4and5inLocalArea

Genotypes	Ages of patients(Years)	Durationof treatment	MedicineNames
1, 2, 3,4, 5	30, 31, 32, 33, 34, 35, 36, 37, 38, 40, 41, 42, 43, 44, 45, 46, 47, 48, 50	12weeks	Sofomac 400mg +Maclinza
3, 5	30, 31, 37, 41, 42, 46	12weeks	Vierof400mg+Ecavir
1, 2, 3, 4, 5	31, 32, 33, 35, 38, 40, 41, 42, 45, 46, 47, 48, 50	12weeks	Zoval400mg+Dakvir
1, 2, 3, 5	30, 31, 32, 34, 35, 36, 37, 38, 40, 41, 42, 43, 45, 47, 48, 49, 50	12weeks	Maclusa400mg + 1000mg
3, 5	33, 35, 36, 38, 40, 42, 43, 46, 47, 48, 50	12weeks	TefodTablet
3, 5	30, 33, 34, 40, 42, 43, 50	12weeks	Sofosbuvir400mg
3	46	12weeks	Ocvir 400mg+Devazo 600mg
3, 5	40, 43	12 weeks	Ecavir
3	38	12 weeks	Zoval 400mg + Daklana
3	48	12 weeks	Vierof 400mg + 100mg

Efficiency Rate of administration of Drugs in Patients

Table 2 is showing patients data with numbers and percentages related to both types of patients, one which had developed SVR in 3 months and other had not.

Table 2: Available direct acting anti-viral drugs in local area with their numbers and percentages of administrationof drugs to Hepatitis C patients

DAA Regimen	All Patients (n = 228)	SVR (n = 206)	No SVR (n = 18)
Sofomac400mg +Maclinza	102 (44.73%)	90 (43.26%)	12 (60%)
Vierof400mg+Ecavir	7 (3.07%)	5 (2.40%)	2 (10%)
Zoval400mg+Dakvir	27 (11.84%)	26 (12.50%)	1 (5%)
Maclusa400mg + 1000mg	49 (21.49%)	48 (23.07%)	1 (5%)
TefodTablet	15 (6.57%)	15 (7.21%)	0 (0%)
Sofosbuvir400mg	10 (4.38%)	9 (4.32%)	1 (5%)
Ocvir 400mg + Devazo 600mg	1 (0.43%)	1 (0.48%)	0 (0%)

Ecavir	2 (0.87%)	2 (0.96%)	0 (0%)
Zoval 400mg + Daklana	1 (0.43%)	1 (0.48%)	0 (0%)
Vierof 400mg + 100mg	10 (4.38%)	9 (4.32%)	1 (5%)
Follow-up lost		4 (1.75%)	

CompleteBloodCount: Highprevalenceofdifferenttestsofcompletebloodcountwasfoundinpost-treatmentpositivegroupascomparedtopost-treatmentnegativegroup,havingdataofpatients with ages 30-50 years, observed after 3 months ofstudy(figure1).

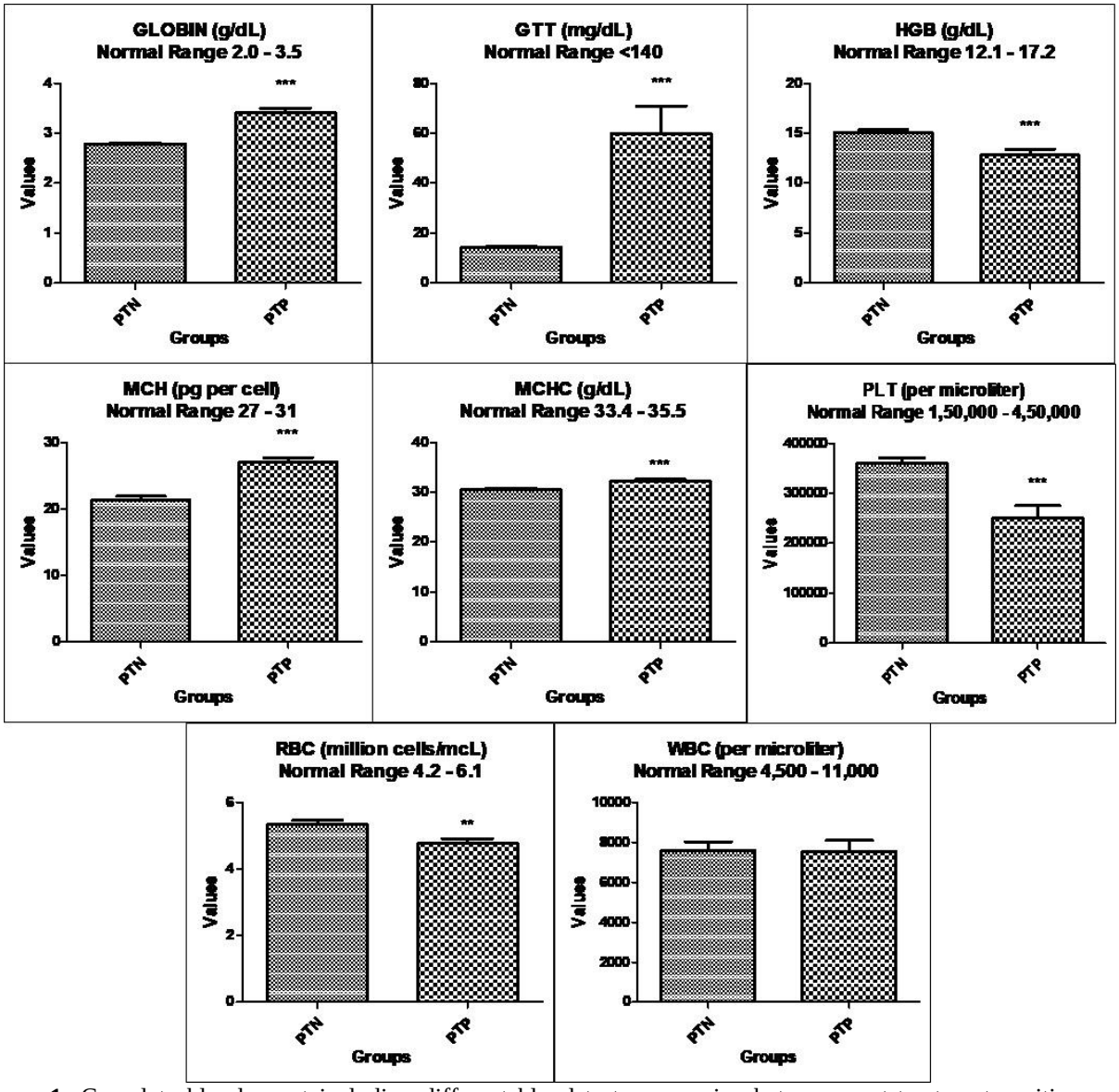

Figure 1: Complete blood count including different blood tests comparisonbetween post-treatment positive and post-treatment negative groups. Datawas calculated as mean ± SEM where p≤0.05 and * shows significancelevelsbetweentwogroups.

Blood Chemistry Panels: High prevalence of different tests ofblood chemistry panels (including renal function tests) was found inpost-treatmentpositivegroupascomparedtopost-treatmentnegative group, having data of patients with ages 30-50 years ofage,observed

after3monthsofstudy(figure2).

Liver Function Tests: High prevalence of liver function tests wasfoundinpost-treatmentpositivegroupascomparedtopost-treatment negative group, having data of patients with ages 30-50yearsof age,observedafter3monthsofstudy(figure3).

PrevalenceofGenotypes,MedicinesandPost-TreatmentEvaluation: In comparison with genotypes 1, 2, 4 and 5, genotype 3patients were more found. Total six kinds of groups of direct actingantiviral drugs were used in study. More than 90% of patients werepost-treatment negative after 3 months of treatment of direct actingantiviral drugs, having data of patients with ages 30-50 years, observedinstudyinduration of 3months(figure 4).

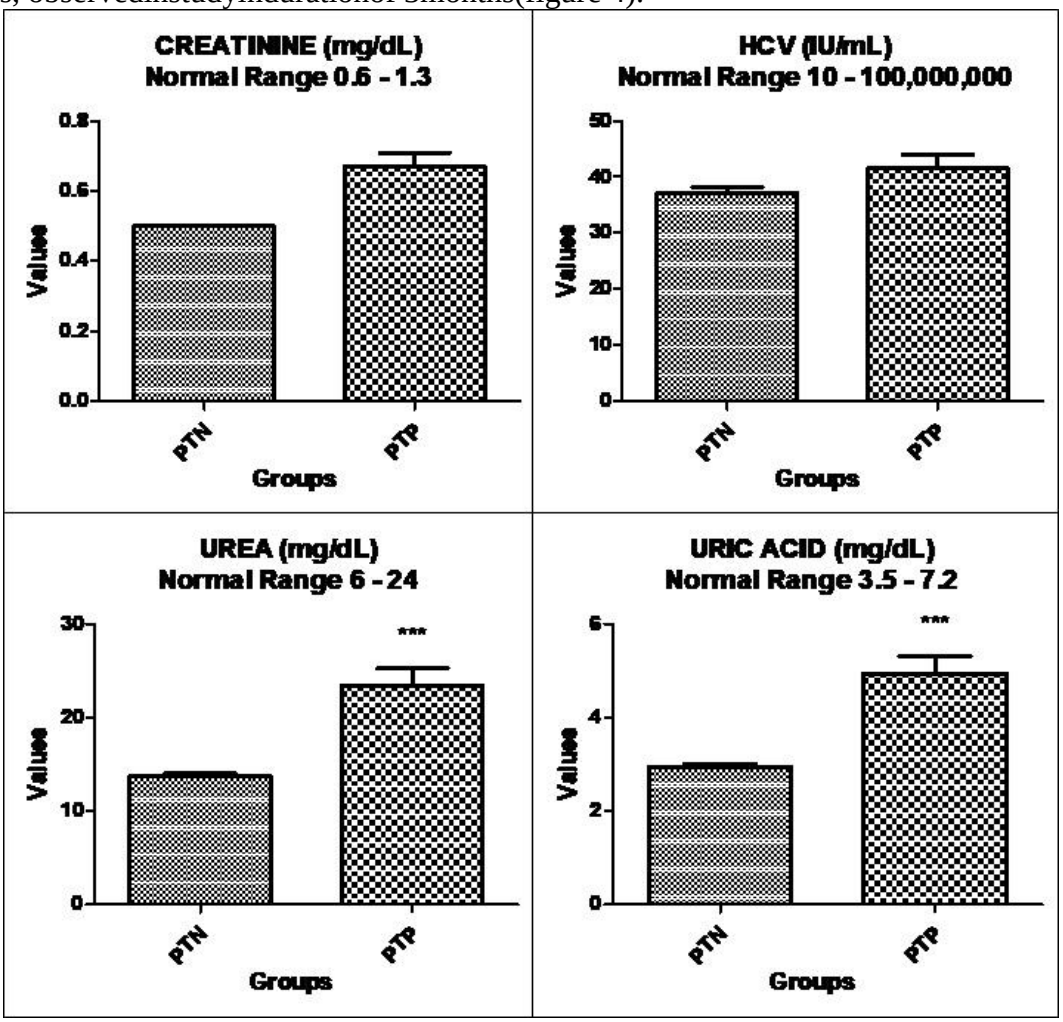

Figure 2: Blood Chemistry Panels including renal function tests comparisonbetween post-treatment positive and post-treatment negative groups. Datawas calculated as mean ± SEM where p≤0.05 and * shows significancelevelsbetweentwogroups.

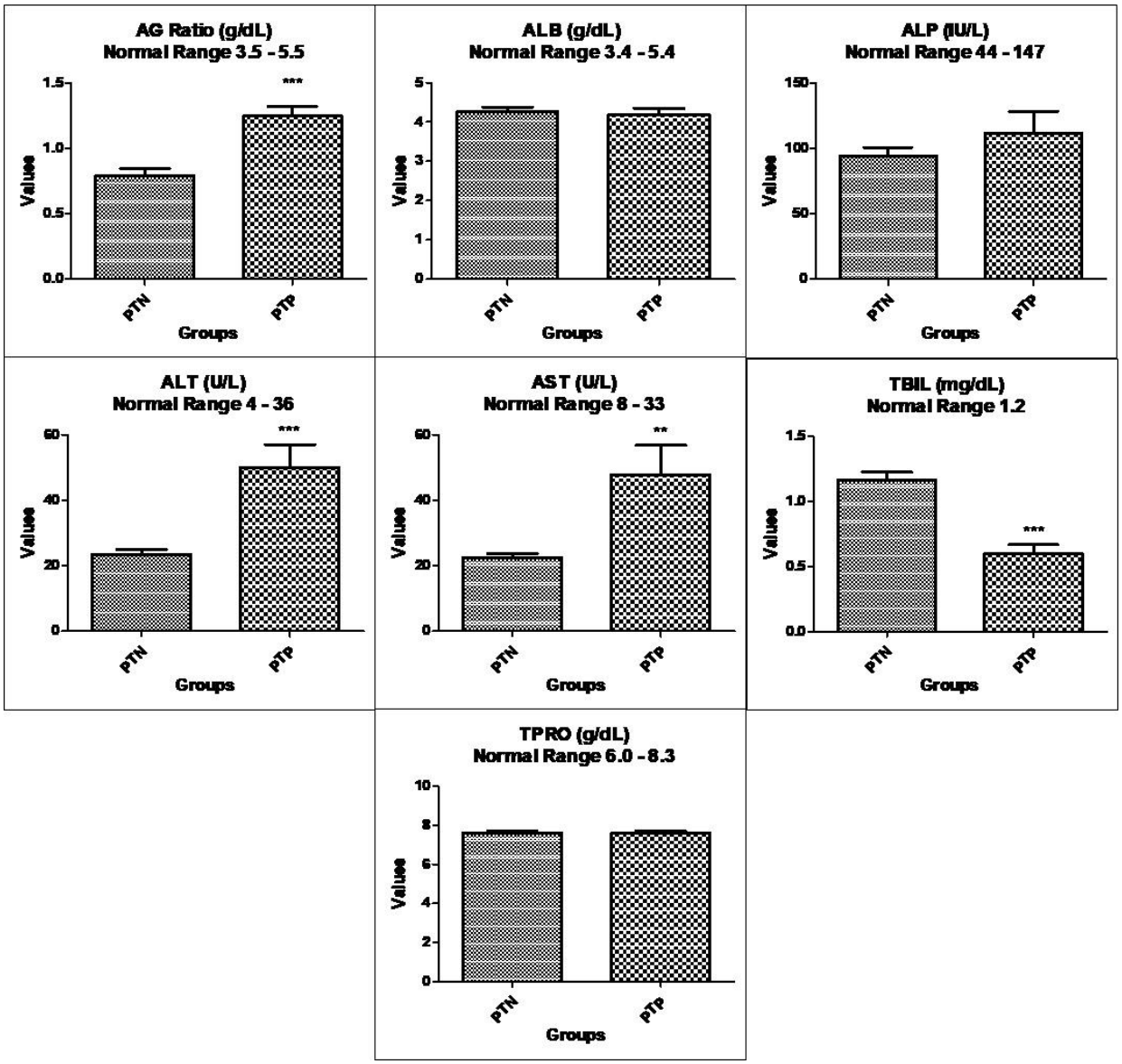

Figure 3: Liver function tests comparison between post-treatment positiveand post-treatment negative groups. Data was calculated as mean ± SEMwherep≤0.05 and*showssignificance levels between two groups.

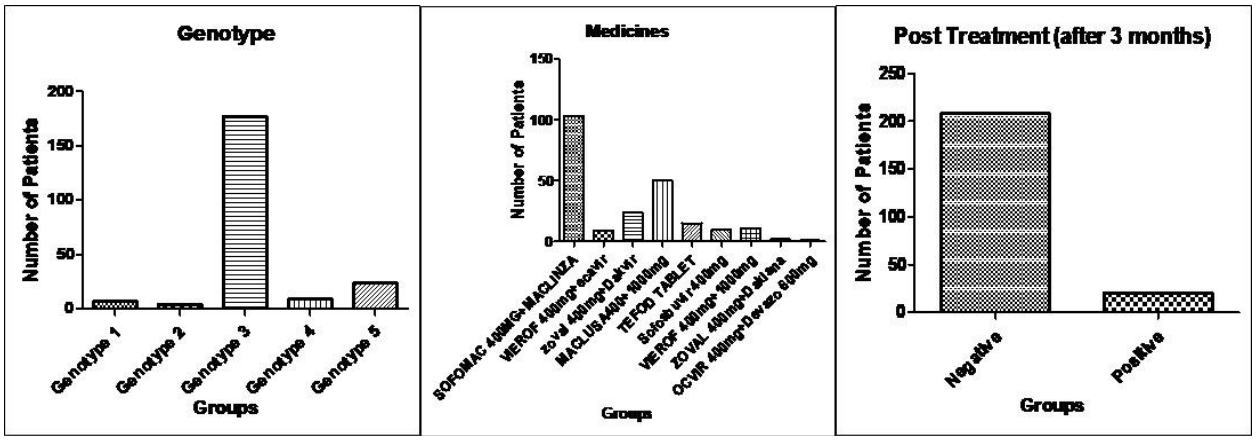

Figure 4: Comparison between post-treatment positive and post-treatmentnegative groups regarding prevalence of

HCV genotypes, kinds direct actingantiviral drugs and number of patients in both groups taking direct actingantiviraldrugsindurationof3 months.

DISCUSSION

Hepatitis C is major cause of liver diseases in which liver is suffered from inflammation to cirrhosis and even cancer (3). Risk of mortality and morbidity determines stage of liver fibrosis during infection of hepatitis C (4). Entry of virus in host cell is necessary for persistence and virulence of disease (5). Virus reside in hepatocytes and cause infection in 80% cells of liver (3).Hepatitis viruses are of different types and their penetration ability varies on basis of genotypes (6).In comparison among all genotypes, least work has done on genotype 3 and therapies of direct acting anti-viral drugs are available for genotype 1 and 2 in most regions of world (7). Hepatitis C patients who developed cirrhosis, 31% were genotype 3 (8). Similarly, 80% patients were genotype 3 in which hepatocellular carcinoma was diagnosed (9). Mechanism of genotype 3 viruses is interlinked with metabolisms of lipids and insulin resistance (10). Much work is required to determine pathogenesis of genotype 3 viruses in human body.

This study has included hepatitis C patients of ages between 30 and 50 years to determine outcome of locally available direct acting anti-viral drugs. Most of hepatitis C studies are based on sustained virological response (SVR), which is usually developed in or after 12 weeks of period. Hence, this study was conducted to measure SVR after treatment of 12 weeks.Using different biological tests and analysis approach, thisstudywasconductedtoinvestigateoutcomeofdirectactingantiviral drugs administration for 3 months in Pakistani hepatitis C patientslocated in Sialkot region with HCV genotypes 1, 2, 3, 4 and 5. ALTand AST levels normally found raised in patients suffering fromacute hepatitis and levels were found reduced after treatment. Generally,plateletsdecreasedanduricacidlevel increased.Theselevelsfluctuatedwithtreatmentandreturnedtonormallimitsindicatingtreatmentsuccess.It was found in currentstudy that an overall recovery rate of more than 90% in 228 patientsof ages between 30 and 50 years andshowedincreasedcurerateascompared to study reported using the older therapy of Peg-IFN +RBV[11]. Most of patients in current studyweregiveneitherSOFOMAC400MG+MACLINZA(n=102)orMACLUSA400+1000mg (n=49)
for3months.Directactingantiviraldrugsadministrationespeciallyingenotype3patients,asmostofpatientsinstudyweregenotype3(n=177), showed SVR as compared to clinical trials conductedearlier (12-15).

CONCLUSION

In conclusion, direct acting antiviral drugs were very effective intreating patients of Sialkot region of Pakistan with HCV genotypes1,2,3, 4and5andanoverallmorethan90%post-treatmentnegativeresultwasfoundafter3monthstreatmentduration in patient's ages between 30 and 50 years.Current study has limitation of observational retrospective nature ofits design. However, more studies can be performed with largeperspective and enough long-term follow-up should be conductedto report more detailed clinical outcomes in HCV patients, alsofurther studies on host genetic factors may explain associationbetweenHCV genotypesandlivercancer/cirrhosisrisks.

Funding: Pak Medical Centre, Sialkot, Pakistan is a famouswelfare organization and provided the facility and concernedfundingtocarryoutrelevant laboratoryworkthere.
Competing interests: Authors have declared that no competinginterestsexistamongthem.

REFERENCES

1. Mansoor G, Muhammad T, Hadi F, Maqbool T, Shakoori T, Malik A. Deranged Haematological Profile and Dyslipidaemiain Diabetes Induced Nephropathy. *J. Pharm. Res. Int.* 2022;34:64-72.
2. Paras H, Tahir M, Mehwish R, et al. Hepatoprotective Effect of Mimosa pudica Leaves Ethanoic Extract in CCl4-Induced Hepatotoxicity.
3. Mailly L, Baumert TF. Hepatitis C virus infection and tight junction proteins: The ties that bind. *Biochimica et Biophysica Acta (BBA)-Biomembranes.* 2020;1862(7):183296.
4. Chan J, Gogela N, Zheng H, et al. Direct-acting antiviral therapy for chronic HCV infection results in liver stiffness regression over 12 months post-treatment. *Digestive Diseases and Sciences.* 2018;63(2):486-492.
5. Ríos-Ocampo WA, Navas M-C, Faber KN, Daemen T, Moshage H. The cellular stress response in hepatitis C virus infection: A balancing act to promote viral persistence and host cell survival. *Virus research.* 2019;263:1-8.
6. Imran HM, Farhan BA, Akram S, et al. A review on advancement towards hepatitis c virus genotyping and novel assays approach for determination of HCV Genotypes.
7. Tada T, Kumada T, Toyoda H, et al. Viral eradication reduces both liver stiffness and steatosis in patients with chronic hepatitis C virus infection who received direct-acting anti-viral therapy. *Alimentary Pharmacology & Therapeutics.* 2018;47(7):1012-1022.
8. Wong YJ, Thurairajah PH, Kumar R, et al. Efficacy and safety of sofosbuvir/velpatasvir in a real-world chronic hepatitis C genotype 3 cohort. *Journal of Gastroenterology and Hepatology.* 2021;36(5):1300-1308.
9. Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, El-Serag HB. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. *Hepatology.* 2020;71(1):44-55.
10. Woyesa SB, Taylor-Robinson AW. Hepatitis C virus infection, genotypes and mechanism of insulin resistance. *Journal of Molecular Pathophysiology.* 2019;8(1):1-13.
11. Nagaoki Y, Imamura M, Aikata H, et al. The risks of hepatocellular carcinoma development after HCV eradication are similar between patients treated with peg-interferon plus ribavirin and direct-acting antiviral therapy. *PLoS One.* 2017;12(8):e0182710.
12. Zeuzem S, Dusheiko GM, Salupere R, et al. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *New England Journal of Medicine.* 2014;370(21):1993-2001.
13. Foster GR, Pianko S, Brown A, et al. Efficacy of sofosbuvir plus ribavirin with or without peginterferon-alfa in patients with hepatitis C virus genotype 3 infection and treatment-experienced patients with cirrhosis and hepatitis C virus genotype 2 infection. *Gastroenterology.* 2015;149(6):1462-1470.
14. Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology.* 2015;61(4):1127-1135.
15. Berden FA, Aaldering BR, Groenewoud H, IntHout J, Kievit W, Drenth JP. Identification of the best direct-acting antiviral regimen for patients with hepatitis C virus genotype 3 infection: a systematic review and network meta-analysis. *Clinical Gastroenterology and Hepatology.* 2017;15(3):349-359.