

Evaluation of Direct-acting Antiviral Drugs for Hepatitis C patients below 30 years age in Sialkot, Pakistan

KASHIF WAQAS¹, BUSHRA NOREEN², ZOYA MUZAFFAR³, FURQAN SABIR⁴, SYED ZEESHAN HAIDER NAQVI¹, JAVED ANVER QURESHI¹, FAHEEM HADI⁵, MUHAMMAD KHAWAR ABBAS⁵

¹Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.

²Department of Biochemistry, Dera Ghazi Khan Medical College, Dera Ghazi Khan, Pakistan.

³Multan Medical and Dental College, Multan, Pakistan.

⁴Molecular Department, Pak Medical Centre, Sialkot, Pakistan.

⁵University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

Correspondence to Dr. Kashif Waqas

ABSTRACT

Background: Infection of Hepatitis C virus (HCV) is major contributor in liver problems, affecting nearly more than 70 million people all over the world. Top ten countries which are affected by Hepatitis, also include Pakistan. Medical science has entered a new era of HCV therapy in elimination of infection and disease is a real possibility. In Asia, Genotype 3 is chronic hepatitis C genotype as compared to other genotypes. Direct-acting antiviral drugs are famous for their high efficacy rate, but less work has done in Asia.

Methods: A follow up research was performed to evaluate 3 months treatment outcome of some direct acting antiviral drugs in 35 patients (26 patients of HCV genotype 3) below 30 years of age using some relevant biological parameters.

Results: A total of 30 patients yielded a pooled SVR of more than 85% after twelve weeks outcome of commonly used antiviral drugs evaluation.

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

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

INTRODUCTION

Chronic HCV affects global population around 180 million that equals 3% of world population (Wen, 2015). Pakistan lacks disinfection controlling mechanisms, management of sewerage and clean water supply deficiencies. For hepatitis C, Pakistan is included in intermediate prevalence zone that are due to unstable healthcare delivery system, political statuses and prevailing socioeconomic conditions that's why, country is unable to tackle further progression of disease (Butt, 2015). Moreover, hepatitis exists in more than one genotype, which make it difficult to treat. 10% patients are present in USA with genotype 3 and this percentage is high in those who are involved in drugs injection (Dar et al., 2021). HCV genotype 3 patients are more prone to liver fibrosis, steatosis and hepatocellular carcinoma as compared to other genotype patients. At present, new acquainted management in medical science give chances of better treatment (Pourhoseingholi, 2014; Adinolfi & Guarrera, 2015). Cirrhotic patients are difficult to treat even by direct acting antiviral drugs (Chan et al., 2017). Consequently, it is evident that HCV is a main concern for healthcare program makers. Luckily, chances of progression to HCC (Hepatocellular Carcinoma) could minimize by 75% through effective management (Kamal-Yanni, 2015; Shahnazarian et al., 2018).

Different protease inhibitors are used for treatment purposes especially in combination and they are effective even in patients of renal failure and liver cirrhosis (Roderburg et al., 2016). Latest treatments have shorter duration therapy, less side effects and increased rate of cure. Different communities are trying to eradicate HCV by 2030. In order to eradicate, first move is to find HCV patients, then second move is to give them cost effective treatment especially in developing countries (Gvinjilia et al., 2016). Major challenge is to manage those HCV patients who may have other fatal diseases along with hepatitis C such as liver failure, kidney problems, HIV and thalassemia, which remain combating in this era. For management of hepatitis C, prevention is equally important as treatment (Hesamizadeh et al., 2016; Wu et al., 2020).

MATERIALS AND METHODS

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 CEPID Smart Cycler by Thermofisher®.

Sampling: These include patient's serum, plasma, EDTA and citrated whole blood. All the samples were subjected to the relevant diagnostic work-up. Peripheral blood was collected from each participant and serum/plasma were stored at - 80°C for molecular assays.

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 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.

Direct Acting Antiviral Drugs: Current direct acting antiviral drugs available in 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 age less than 30 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 age range of 07 – 29 years were enrolled in this study. Participants with high ALT level (1.5 times more than 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 agree 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 co-infected with HBV. Pregnant females were not enrolled in this study. Patients having either extra hepatic malignancy or hepatocellular carcinoma.

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

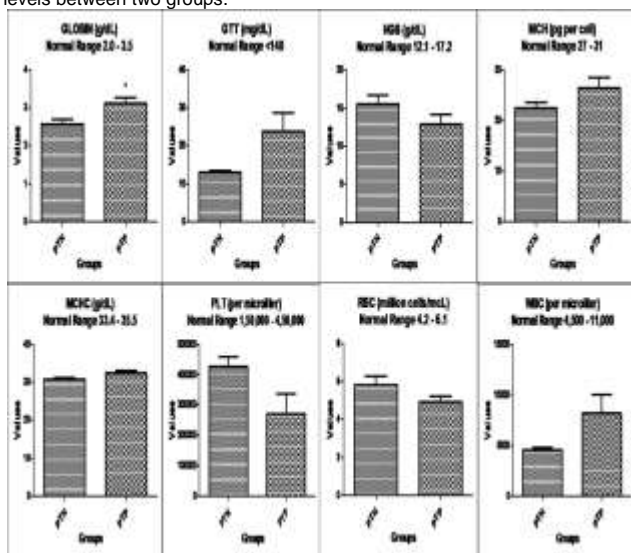
Table 1: Current treatment options for genotypes 1, 2, 3 and 5 in Local Area

Genotypes	Ages of patients (Yrs)	Duration of treatment	Medicine Names
3, 5	07, 20, 23, 24, 25, 26	12 weeks	Sofomac 400mg + Maclinza
3	18, 23, 27	12 weeks	Vierof 400mg + Ecavir
1, 3	13, 26, 29	12 weeks	Zoval 400mg + Dakvir
2, 3	18, 21, 22, 24, 25, 26, 28, 29	12 weeks	MACLUSA 400mg + 1000mg
3, 5	28, 29	12 weeks	Tefod Tablet
3	27	12 weeks	Sofosbuvir 400mg

RESULTS

Complete Blood Count: High prevalence of different tests of complete blood count was found in post-treatment positive group as compared to post-treatment negative group, having data of patients with less than 30 years of age, observed after 3 months of study (Fig. 1).

Figure 1: Complete blood count including different blood tests comparison between post-treatment positive and post-treatment negative groups. Data was calculated as mean ± SEM where p<0.05 and * shows significance levels between two groups.



Blood Chemistry Panels: High prevalence of different tests of blood chemistry panels (including renal function tests) was found in post-treatment positive group as compared to post-treatment negative group, having data of patients with less than 30 years of age, observed after 3 months of study (figure 2).

Liver Function Tests: High prevalence of liver function tests was found in post-treatment positive group as compared to post-treatment negative group, having data of patients with less than 30 years of age, observed after 3 months of study (figure 3).

Prevalence of Genotypes, Medicines and Post-Treatment Evaluation: In comparison with genotypes 1, 2 and 5, genotype 3 patients were more found. Total six kinds of groups of direct acting antiviral drugs were used in study. More than 85% of patients were post-treatment negative after 3 months of treatment of direct acting antiviral drugs, having data of patients with less than 30 years of age, observed in study in duration of 3 months (figure 4).

Received on 24-04-2022
Accepted on 11-08-2022

Figure 2: Blood Chemistry Panels including renal function tests comparison between post-treatment positive and post-treatment negative groups. Data was calculated as mean ± SEM where p<0.05 and * shows significance levels between two groups.

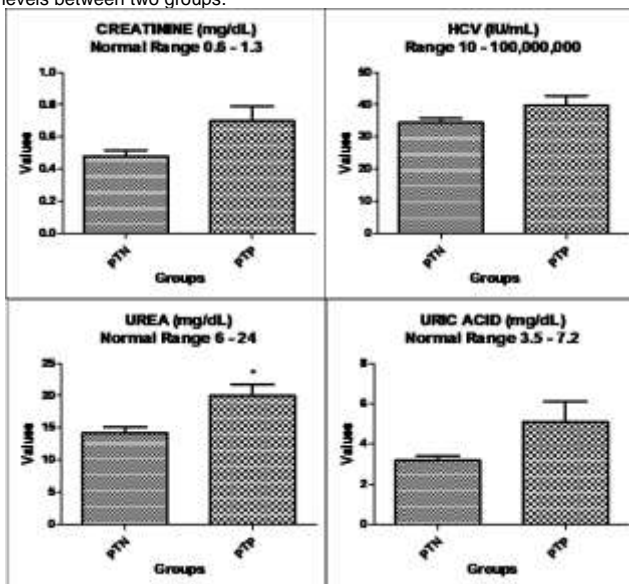


Figure 3: Liver function tests comparison between post-treatment positive and post-treatment negative groups. Data was calculated as mean ± SEM where p<0.05 and * shows significance levels between two groups.

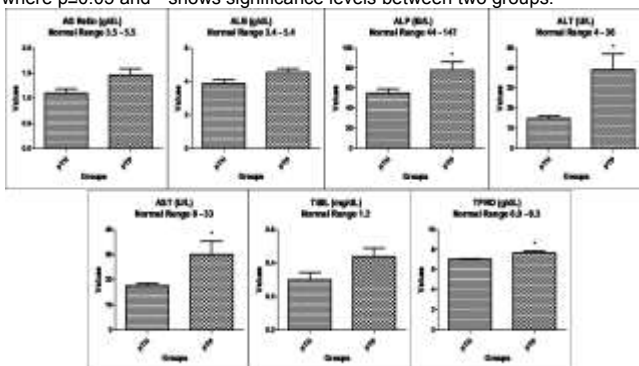
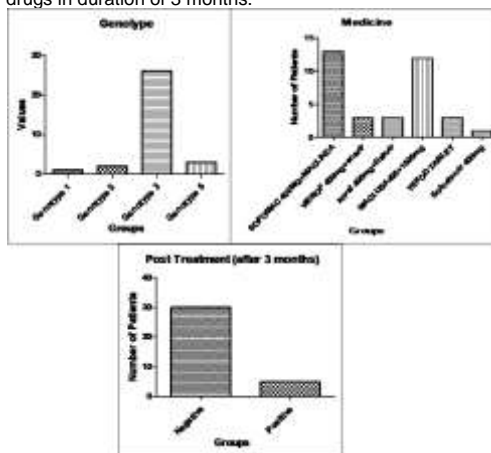


Figure 4: Comparison between post-treatment positive and post-treatment negative groups regarding prevalence of HCV genotypes, kinds direct acting antiviral drugs and number of patients in both groups taking direct acting antiviral drugs in duration of 3 months.



DISCUSSION

Development of direct acting antiviral drugs improved outcome of chronic HCV patients. Penetration capability of virus in host is a key determinant of its virulence and persistence. For initiation of infection, cell-free entry is vital in which virions enter liver via blood and causes establishment of infection in adjacent hepatocytes (Xiao et al., 2014). It is observed that there are direct acting antiviral drugs therapies available for genotypes 1 and 2, but less work has done on genotype 3 patients regarding their followup and outcome evaluation.

Genotype 3 prevalence is more common in South Asia (especially Pakistan and India), West Asia (Iran) and South-East Asia (Myanmar) as compared to Taiwan, Korea and Japan, due to relatively low frequency in these countries (Wei et al., 2018). It was found in a study that genotype 3 patients were 80% more chances to develop hepatocellular carcinoma and 31% more chances to develop cirrhosis, in comparison with genotype 1 patients. However, HCV genotype 2 patients have less risk of development of cirrhosis and liver cancer as compared to genotype 1 (Kanwal et al., 2014). It is also observed that there is close relation between HCV genotypes and their penetration capability within host liver. That's why, some genotypes are able to infect severely and some are not (Xiao et al., 2014). Genetic studies (not included in current study) may reveal association between HCV genotypes and liver cancer/cirrhosis risks. For instance, a study reported relation between HCV genotypes 2/3 and C allele, conducted in European population (Kanwal et al., 2014). In a study, antiviral strategy was found effective in genotype 1 in cell culture and animal models, but still there was no data against non-genotype 1 work (Xiao et al., 2014). HCV genotype 3 has been expected to prove more difficult in cure, though new HCV treatments have evolved. Because of genotype 3 having distinct characteristics like insulin resistance and modifications in the lipid metabolism, it may partly show lesser cure responses. The population that seems to develop fast hepatic deterioration and increased frequency of liver cancer, have to require evolving novel strategies to gain better outcome. Multiple potential aspects of HCV genotype 3 pathogenesis are still unclear.

Age is much related with progression of liver fibrosis in HCV patients (Kanwal et al., 2014), that's why, this study included patients of less than 30 years of age and is divided into two groups which is based on treatment outcome, after administration of direct acting antiviral drugs for 3 months period. It is observed that HCV genotype 3 patients are more common in old age as compared to genotype 1 (Kanwal et al., 2014), but in current study, genotype 3 patients were found more common in patients below 30 years of age as compared to genotypes 1, 2 and 5.

Using different biological tests and analysis approach, this study was conducted to investigate outcome of direct acting antiviral drugs administration for 3 months in Pakistani patients located in Sialkot region with HCV genotypes 1, 2, 3 and 5. ALT and AST levels normally found raised in patients suffering from acute hepatitis and levels decrease during treatment. Generally, platelets decrease, and uric acid rise initially. These levels fluctuate with treatment and return to normal limits indicating treatment success. Identification of significant biomarkers like HbA1c (glycated hemoglobin) and AFP (alpha fetoprotein) that change in response to treatment in hepatitis may help the clinicians in deciding duration of treatment. It was found in current study that an overall recovery rate of more than 85% in 35 patients below 30 years of age, which showed increased cure rate as compared to study reported using the older therapy of Peg-IFN + RBV (Goossens & Negro, 2014). Most of patients in current study were given either SOFOMAC 400MG+MACLINZA (n=13) or MACLUSA400+1000mg (n=12) for 3 months.

Direct acting antiviral drugs administration especially in genotype 3 patients, as most of patients in study were genotype 3

(n=26), showed high SVR as compared to clinical trials conducted earlier (Zeuzem et al., 2014; Foster et al., 2015; Nelson et al., 2015; Berden et al., 2017).

CONCLUSION

In conclusion, direct acting antiviral drugs were very effective in treating patients of Sialkot region of Pakistan with HCV genotypes 1, 2, 3 and 5 and an overall more than 85% post-treatment negative result was found after 3 months treatment duration. Current study has limitation of observational retrospective nature of its design. However, more studies can be performed with large perspective and enough long-term follow-up should be conducted to report more detailed clinical outcomes in HCV patients, also further studies on host genetic factors may explain association between HCV genotypes and liver cancer/ cirrhosis risks.

Funding: Pak Medical Centre, Sialkot, Pakistan is a famous welfare organization and provided the facility and concerned funding to carry out relevant laboratory work there.

Competing interests: Authors have declared that no competing interests exist among them.

REFERENCES

- Adinolfi, L. E., & Guerrera, B. (2015). All-oral interferon-free treatments: The end of hepatitis C virus story, the dream and the reality. *World journal of hepatology*, 7(22), 2363–2368. <https://doi.org/10.4254/wjh.v7.i22.2363>.
- Berden, F. A., Aaldering, B. R., Groenewoud, H., et al. (2017). Identification of the best direct-acting antiviral regimen for patients with hepatitis C virus genotype 3 infection: a systematic review and network meta-analysis. *Clin Gastroenterol Hepatol*, 15, 349–59.
- Butt, A. S. (2015). Epidemiology of viral hepatitis and liver diseases in Pakistan. *Euroasian J Hepato-Gastroenterol*, 5(1), 43–48.
- Chan, A., Patel, K., & Naggie, S. (2017). Genotype 3 infection: the last stand of hepatitis C virus. *Drugs*, 77(2), 131–144.
- Dar, G. A., Yattoo, G. N., Gulzar, G. M., Sodhi, J. S., Gorka, S., & Laway, M. A. (2021). Treatment of Chronic Hepatitis C Genotype 3 With Ledipasvir and Sofosbuvir: An Observational Study. *Journal of Clinical and Experimental Hepatology*, 11(2), 227–231.
- Foster, G. R., Pianko, S., Brown, A., et al. (2015). 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*, 149, 1462–70.
- Goossens, N., & Negro, F. (2014). Is genotype 3 of the hepatitis C virus the new villain? *Hepatology*, 59, 2403–12.
- Gvinjilia, L., Nasrullah, M., Sergeenko, D., Tsertsvadze, T., Kamkamidze, G., Butashvili, M., ... & Averhoff, F. (2016). National progress toward hepatitis C elimination—Georgia, 2015–2016. *Morbidity and Mortality Weekly Report*, 65(41), 1132–1135.
- Hesamizadeh, K., Sharafi, H., Rezaee-Zavareh, M. S., Behnava, B., & Alavian, S. M. (2016). Next steps toward eradication of hepatitis C in the era of direct acting antivirals. *Hepatitis monthly*, 16(4).
- Kamal-Yanni, M. (2015). Hepatitis C drug affordability. *The Lancet Global Health*, 3(2), e73–e74.
- Kanwal, F., Kramer, J. R., Ilyas, J., Duan, Z., & El-Serag, H. B., ... (2014). HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. *Hepatology*, 60, 98–105.
- Nelson, D. R., Cooper, J. N., Lalezari, J. P., et al. (2015). All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*, 61, 1127–35.
- Pourhoseingholi, M. A., Ashtari, S., & Alavian, S. M. (2014). Sofosbuvir vs. Combination of Pegylated Interferon and Ribavirin; How Much Shall Pay for Iranian Patients?. *Hepatitis monthly*, 14(11).
- Roderburg, C., Tacke, F., & Trautwein, C. (2016). Antiviral therapy in patients with viral hepatitis and hepatocellular carcinoma: indications and prognosis. *Visceral medicine*, 32(2), 121–126.
- Shahnazarian, V., Ramai, D., Reddy, M., & Mohanty, S. (2018). Hepatitis C virus genotype 3: clinical features, current and emerging viral inhibitors, future challenges. *Annals of gastroenterology*, 31(5), 541.
- Wei, B., Ji, F., Yeo, Y. H., et al. (2018). Systematic review and meta-analysis: real-world effectiveness of direct-acting antiviral therapies in chronic hepatitis C virus genotype 3 in Asia. *BMJ Open Gastro*, 5:e000209.
- Wen, Y., Zheng, Y. X., & Tan, D. M. (2015). A comprehensive long-term prognosis of chronic hepatitis C patients with antiviral therapy: a meta-analysis of studies from 2008 to 2014. *Hepatitis monthly*, 15(5).
- Wu, N., Rao, H. Y., Yang, W. B., Gao, Z. L., Yang, R. F., Fei, R., ... & Wei, L. (2020). Impact of hepatitis C virus genotype 3 on liver disease progression in a Chinese national cohort. *Chinese medical journal*, 133(3), 253.
- Xiao, F., Fofana, I., Heydmann, L., Barth, H., Soulier, E., Habersetzer, F., et al. (2014). Hepatitis C virus cell-cell transmission and resistance to direct-acting antiviral agents. *PLoS Pathog*, 10(5), e1004128.
- Zeuzem, S., Dusheiko, G. M., Salupere, R., et al. (2014). Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med*, 370, 1993–2001.