www.ajlifesciences.com / ISSN: 2663-1040 *AJ Life Sci. 2021, 4 (1): 73-79* **Research Article**

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.4.1.9

Assessment of Detection and Treatment Response of Hepatitis C viral Infection in District Mardan Pakistan

Atif Kamil*[1], Aurang Zeb[1,5], Farooq Hussain[2], Muhsin Jamal[3], Azmat Ali[2], Muhammad Shah[2], Ayaz Ahmad[1], Jawad Khan[1], Faheem Anwar[1], Nabila[4]

[1]*Department of Biotechnology, Abdul Wali Khan University Mardan, KPK, Pakistan*
[2]*Departemnt of Physical Education and Sports, Abdul Wali Khan University Mardan, KPK, Pakistan*
[3]*Department of Microbiology, Abdul Wali Khan University Mardan, KPK, Pakistan*
[4]*Department of Plant Sciences, Quaid-i-e Azam University Islamabad, Pakistan*
[5]*School of Biology and Basic Medical Sciences, Soochow University China*

Abstract

Hepatitis C virus is known to be major public health issue around the globe. The aim was to investigate the treatment and detection of HCV infection in general population of District Mardan. Presently no standard treatment is available for the cure of Hepatitis C viral infection, attributed possibly to the hyper variations in HCV genome, expressing several distinct HCV genotypes. For a period of 5 months (June 2016 - November 2016), 270 suspected individuals visited the main hospital of Mardan Medical complex (MMC), were interviewed. Among them 100 individuals were diagnosed by ELISA and PCR for HCV detection at the diagnostic laboratory of MMC. A total of 170 HCV patients were treated at the MMC, with oral medicine or interferon vaccines and the response were monitored by PCR after treatments. Our result showed that high sensitivity for genome-based PCR detection of HCV in comparison to viral coat protein detection by ELISA. The assessment of treatment strategies for HCV showed high response for presently available medicines i.e., Sovaldi, Sofiget, Sofohil, Ocvir and sofosbuvir in comparison to interferon and pig interferon. The accurate and early diagnosis of the HCV infection is crucial for effective treatment strategies. Thereby PCR detection should be the proffered method for specific and accurate detection of HCV infection. The standard medicine available presently proved better treatment method for HCV infection in comparison to interferon vaccination.

Key words: Vaccine, Medication, Sovaldi, Mardan, Interferon, Sofohil, HCV

Article Info:
Received:
February 27, 2021
Received Revised:
March 30, 2021
Accepted:
March 31, 2021
Available online:
September 11, 2021

**Corresponding Author:*
atifkamil@awkum.edu.pk

1. INTRODUCTION

Hepatitis C virus is a 50 nm envelope RNA virus that belongs to the Flaviviridae family and was first discovered in 1080. Hepatitis is a chronic disease. Hepatitis C virus (HCV) infection causes the highest number of deaths worldwide each year[1,2]. HCV is one of the leading causes of illness and death worldwide. It reflects the high genetic diversity that is a global phenomenon Features regional variations in genotype patterns. About 3% of the world population is forever infected with HCV, which is crucial Causes of liver failure such as liver cirrhosis, liver fibrosis and Hepatocellular carcinoma and vital liver function Patient (However, this death rate is comparable with the 1.5 million deaths caused by HIV and 1.2 million deaths resulting from tuberculosis or malaria every year [3,4]. Worldwide the predictable value of HCV cirrhosis is about 25%[5]. Primarily HCV transmission route is blood transfusion, unsafe injection practices and sex etc. HCV causes long-lasting infection in about 80% of its victims. The HCV infection is detected by screening high hazard collections for anti-HCV molecules (antibodies to HCV) in the patient sample. The researchers are trying to get rid of this life threading diseases by applying different vaccines, herbal treatment and now medication. However, due to high degree of genetic variations in HCV genome as well as mutation rates variability reported in the different genomic regions reduced the rate of successful treatment[6,7]. Several different isolates of HCV genomic RNA as well different proteomic structural domain for HCV coat have been reported[8,9]. Several oral medicine and few vaccines are available for the treatment of HCV infection, still assessment is needed for understanding the therapeutic success of vaccines in comparison to oral medicines[10, 12]. At MMC Mardan, the most commonly prescribed medicines were, Sovaldi (Sofosbuvir), Sofohil, Ocvir and Sofiget and the vaccines used were interferon and pig interferon for the treatment of HCV infected patients. The current study was aimed to explore the present diagnostic approaches as well as treatment strategies for HCV infection, at MMC Mardan located in the second largest city of KPK, Pakistan.

2. MATERIALS AND METHODS

2.1 Designing of the study

District Mardan of Khyber Pakhtunkhwa (Pakistan) was selected for this cross-sectional study and the general population is focused mainly because of the high rate of HCV infected patients in the selected area were reporting to nearby the hospitals.

2.2 Criteria for the included peoples

All ages of males and females (adults) population, different races and ethnic collections that belong to District Mardan, KP, Pakistan, were included in this study. All patients visited OPD for check-up or admitted to MMC, Mardan from June 27th, 2016 to 22nd November 2016wereincluded in this study. Total 270 individuals suspected of HCV infection were interviewed with standard questionnaire, for detailed history. Only 100 individuals were screened for HCV infection by ELISA and PCR at MMC diagnostic laboratory. Blood samples were collected from patients under non profitability convenience. A total of 170 HCV patients were treated at MMC with oral medicines or vaccinations. The treatment responses were monitored by PCR screening after the treatments.

2.3 Sample collection

Each individual was exposed to laboratory assessments (tests) after history and physical check-up. Three (3) ml of vein blood sample was collected under strict germ-free environment in a sterile not reusable plastic injection. The fresh blood was transferred into a plastic bottle without anticoagulant and kept at room temperature for clotting. For extracting serum, the clotted blood was centrifuged. Afterwards, the samples were stored at -20 ºC till further use.

2.4 Detection of HCV infection by ELISA

Enzyme Linked Immunosorbent Assay (ELISA)was performed using Architect Anti-HCV Reagent kit- Abbott according to Manufacturer´s protocol. The patient serum was used for qualitative detection of antibody to HCV (anti-HCV protein) because the micro-wells are pre-coated with recombinant HCV encoded antigen as the solid phase. Those samples were considered negative where the absorbance value was less than the cut off value (01), however the samples were considered reactive where the absorbance value were greater

than cut off value. The reactive samples were repeated twice and trice to obtain the possible accurate result.

2.5 Detection of HCV infection by Real-Time Polymerize Chain reaction (PCR)

For Real-Time PCR total RNA was extracted from 200 µl of serum using QIAamp® 96 Virus QIAcube® HT Kit (Catalogue No. 57731), following manufacturer's instructions. HCV RNA was amplified by RT-PCR from highly conserved region of the viral genome, i.e. 5'-untranslated regions (5'-UTR), using artus® HCV QS-RGQ Kit - Qiagen (Catalogue No. 4518366) in duplicates. The amplified product (240bp) is detected via fluorescent dyes, linked to oligonucleotide probes that bind specifically to the amplified product. The fluorescence intensities were monitored during the PCR run (in real-time) for detection and quantification of the accumulating product in PCR tubes. Target sequence amplification was done by 30 cycles with denaturation at 95ºC for 25s, primer annealing at 50ºC for 35s and extension at 68ºC for 2min and 30s. Final extension was carried out at 68ºC for 9min and 30s. For qualitative detection, the ct value above 34 was considered negative whereas ct value above 34 was considered positive. For quantitative analysis we considered measurement above the 25 iu/ml as positive and value measuring bellow 25 iu/ml as negative.

2.6 Analysis of Data

Tall the collected data were processed and analyzed by statistical methods like SPSS-11.0 for windows 7 by version 5 of Graph Pad Prism. The calculated ratio and percentages were of different variables.

3. RESULTS AND DISCUSSIONS

3.1 Detection of HCV infected patients

The 100 individuals visited the diagnostic laboratory of MMC Mardan, were analyzed by ELISA as well as PCR technique for the detection of HCV infection. The ELISA results of these 100 individuals revealed 87 individuals infected with HCV (table.1 shows a positive ELISA result), whereas 13 individuals were found free from HCV infection. However, PCR method reported 72 individuals infected with HCV (figure.2 shows a positive PCR result) and 28 individuals were found free from HVC infection. These findings showed the high specificity for the genome detection method of PCR, in comparison to antibody-based protein detection ELISA technique (figure.1).

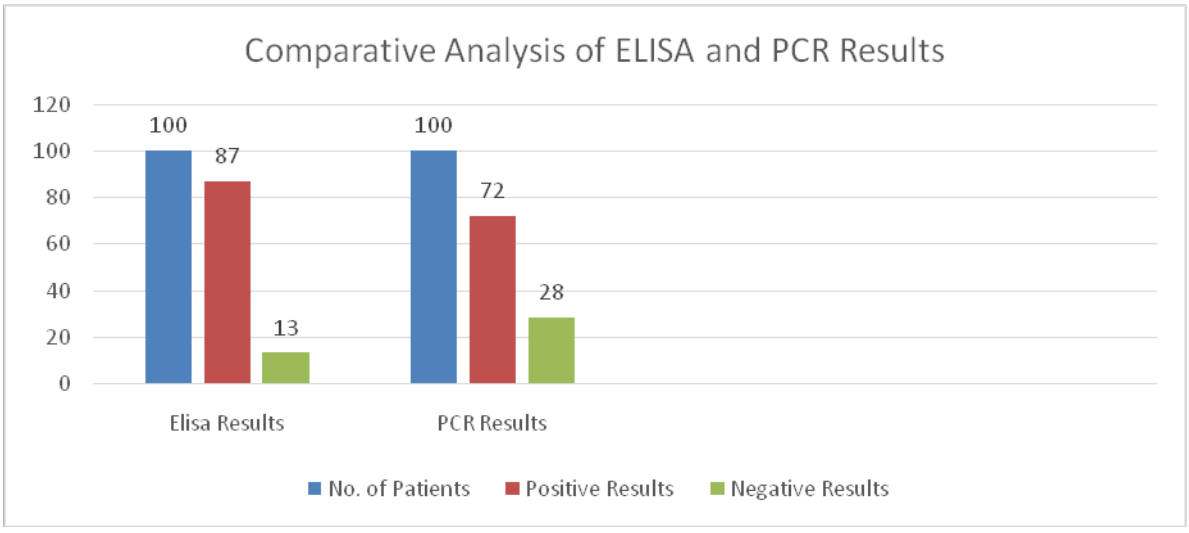

Figure.1: Comparative Analysis of ELISA and PCR Method

Table.1. ELISA Positive Cases of HCV

TEST	RESULT
CUT OFF VALUE	1.00
HBS Ag	
HCV AB	9.6 positive
REMARKS	Sample was found positive for HCV

Result: HCV RNA Detected, COPIES: 535,839 iu/ML

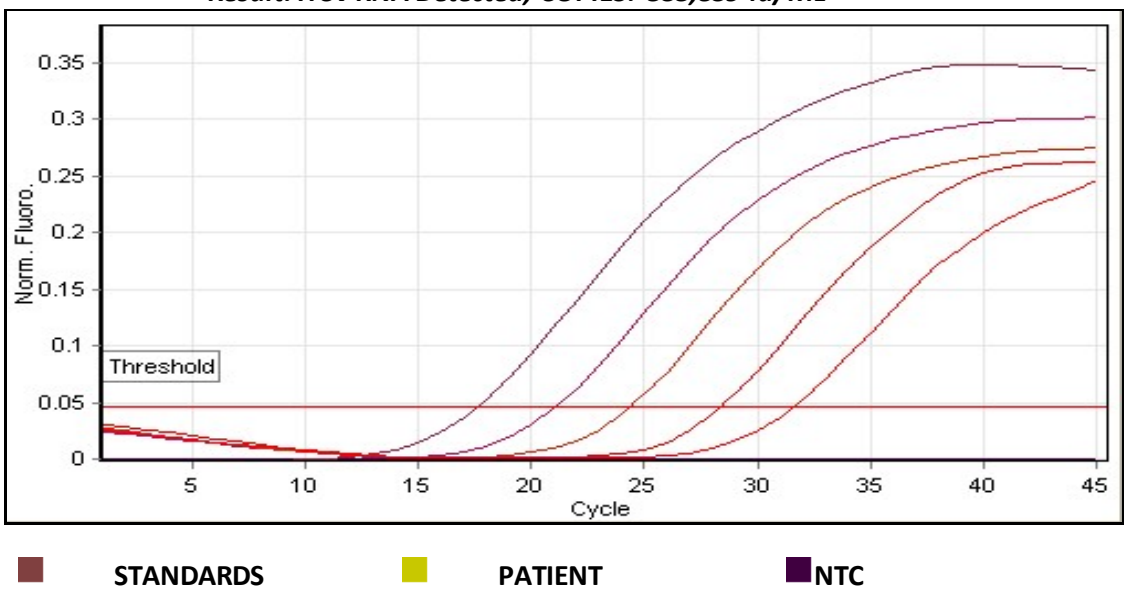

Figure.2: Representation of the PCR positive cases of HC infection at MMC Mardan.

3.2 The response of medicines in comparison to vaccines for HCV patients

HCV infected individuals (170) visited MMC Mardan, were treated with either vaccines or medicines. The two verities of vaccines i.e. interferon and pig interferon and four different medicines i.e. Sovaldi (sofosbuvir from Feroz Sons), Sofohil (from Hilton) Ocvir (from Gabr and Alghadir) and Sofiget (from Getz) were used for the treatment of HCV infected patients. All these four different oral medicines showed very effective response in the treatment of HCV patients when analyzed by PCR after treatment (figure.3). Pig-interferon also showed good response in treatment of HCV patients; however, interferon did not report similar effective response in the treatment of HCV patients (illustrated in table.2).

Table. 2: Percentage response of medicines and vaccines after treatment

S. No	Treatment Strategies of HCV Patients			PCR Negative	PCR Positive	Percentage (%)
1	Vaccination	Interferon	30 samples	23	7	76.7%
2		Pig-Interferon	30 samples	28	2	93.3%
3	Medication	Sovaldi	20 samples	20	0	100%
4		Sofohil	25 samples	24	1	96%
5		Ocvir	25 samples	25	0	100%
6		Sofiget	40 samples	39	1	97.5%

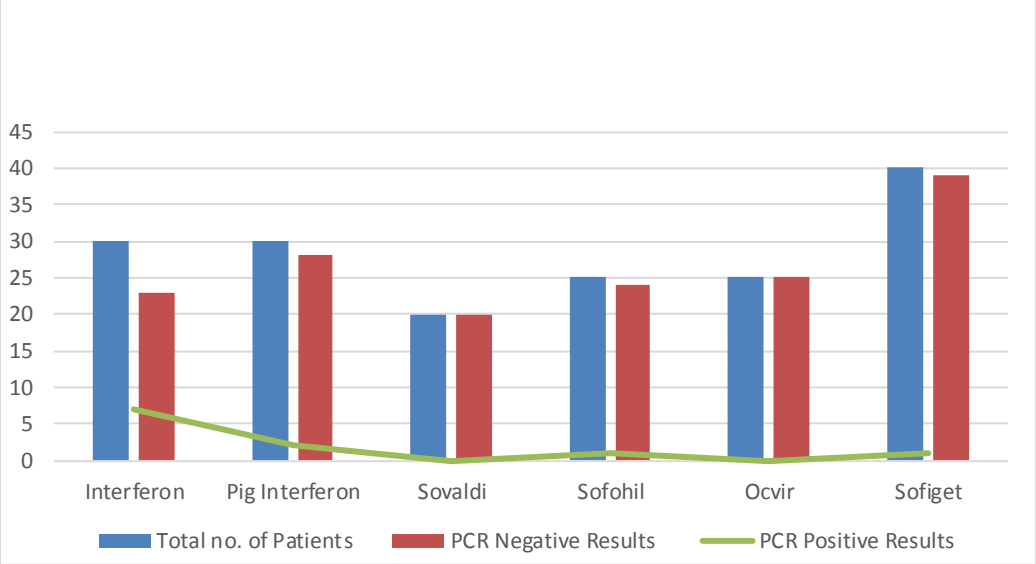

Figure.3: The Response of vaccines and medicine in HCV patients visited MMC Mardan.

Hepatitis C Virus is the leading cause of morbidity and mortality throughout the world[15]. HCV prevalence previously reported in overall Pakistan was 4.7%[16], 4.95%[17], and then 4.87%[18]. One of the studies estimated that in Pakistan about 6.8% adults are infected by HCV infections. Thereby most patients reporting to this hospital are from local areas like same area as Mardan (12.55%, 1.2%) and Charsadda[13,14,15]. The high prevalence ratio of HCV infection in Mardan could be attributed to poor hygiene, less awareness and non-serious attention of clinical institutes. The patients of HCV both in private and public health institutes are not properly diagnosed due to lake of high throughput techniques[21]. The patients are not provided with proper information and guidance for the adequate use of medicines and other cures. Majority of the

society is unaware about transmission of HCV and its contagious ability thus does not carry out any precautions. The knowledge and awareness about transmission is very important in a society that they may take precautions and defend themselves against HCV infection. For investigation of HCV infection has close link with accurate and early detection. The detection of HCV has improved with the advances in the field of molecular diagnostics, specially the two well known methods ELISA and PCR. ELISA is protein detection serological method routinely used in clinics and hospitals, whereas PCR[19]. The treatment of HCV infection has improved by availability of several oral medicines as well as vaccines. *Jamil et al., 2018* research project reported that oral medications available in the market are very effective against HCV infection, specially sofosbuvir and ovir, although interferon and pig interferon vaccines also showed good results for treatment of HCV patients.[20,21] The diagnosis of HCV infected individuals revealed that PCR detection is more accurate and specific in comparison to ELISA[19].ELISA is routinely use method of detection for HCV and several other diseases in clinical institutes and hospitals. PCR detection method is preferred over ELISA due to the highly specified detection nature and identification of PCR technique. The sensitivity nature of PCR makes it the first choice in early diagnosis of several genetic disorders. In the current study we reported 32.22% patients positive for HVC infection through ELISA with 270 samples size. Similar results were reported in another study in Pakistan for ELISA detection of HCV infection i.e.,22.48% positive HVC infection and 0.7% co-infection with Hbs with 845 samples size[10]. Our PCR detection of the same samples showed 27% HCV infected patients and a recent study reported 30% HCV infected patients with similar sample size from district Mardan[11].The PCR diagnosed 170 patients of HCV infection were treated with different medicines i.e., sofohil, sofiget, sovaldi and ocvir, showed better results in comparison to the treatment with interferon and pig interferon vaccines. Thereby highly specified vaccines are required for improved treatment of HCV infection. Thus, currently oral medicine treatment could cure almost all six types of HCV genotypes and the available interferon and pig interferon vaccines could not trigger specified immune responses against these genotypes. Thereby suggest further instigations for more highly specified vaccines against all genotypes of HCV.

4. CONCLUSIONS

According to our study that showed that medicines treatment of HCV positive patients is better than vaccines treatment. The vaccines may have complications in some patients. Thereby the vaccines complications could be further investigated. The Linkage of genotyping with vaccine treatment could be explored further, to suggest the use of specific vaccine verities for certain patients.

5. ACKNOWLEDGMENT

The authors are thankful to the Mardan Medical Complex for helping in execution of this research work and allowing access to its diagnostic laboratories. The other financial requirements were supported by Higher Education Commission (HEC) of Pakistan with project Start-up Research Grant programme (SRGP).

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. REFERENCES

1. Mostafa, A., Shimakawa, Y., Medhat, A., Mikhail, N. N., Chesnais, C. B., Arafa, N., ... & Fontanet, A. Excess mortality rate associated with hepatitis C virus infection: A community-based cohort study in rural Egypt. *Journal of hepatology*. 2016. *64*(6), 1240-1246.
2. Taherkhani, R., & Farshadpour, F. Global elimination of hepatitis C virus infection: Progresses and the remaining challenges. *World journal of hepatology*.2017 *9*(33), 1239.
3. Wahid, B., Waqar, M., Rasool, N., Rehman, Z., Saeed, J., Wasim, M., ... & Idrees, M. Recent trends in molecular epidemiology of Hepatitis C virus in Mardan, KPK Pakistan. *Infection, Genetics and Evolution*2018. *66*, 66-71.
4. Din, M., Anwar, F., Ali, M., Yousaf, M., & Ahmad, B. Chemiluminescent-microparticle-immunoassay-based detection and prevalence of human immunodeficiency virus infection in Islamabad, Pakistan. *Archives of Virology*, 2020. 1-6.
5. Alter, M. J. Epidemiology of hepatitis C virus infection. *World journal of gastroenterology: WJG*. 2017 .*13*(17), 2436.

6. Martell, M., Esteban, J. I., Quer, J., Genesca, J., Weiner, A., Esteban, R., ... & Gomez, J. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasi species nature of HCV genome distribution. *Journal of virology*.1992 *66*(5), 3225-3229.
7. Pawlotsky, J. M. Hepatitis C virus population dynamics during infection. *Quasispecies: Concept and Implications for Virology*. 2006 261-284.
8. Wang C, Sarnow P, Siddiqui A. Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. Journal of virology. 1993; 67:3338-3344.
9. Basit A, Rahim K, Ahmad I, Shafiqe M, Mushtaq S, Khan I, Shaheen H. Prevalence of hepatitis B and C infection in Pakistan. J. Inf. Mol. Biol. 2014; 2(3): 35 – 38.
10. Waqar M, Khan AU, Ali A, Wasim M, Muhammad Idrees M, Zobia Ismail Z, Noor AA, Noorul Akbar N, Bano S, Khan MA, Khan RK. Prevalence and Molecular Determination of Hepatitis C Infection in Khyber Pakhtunkhwa, Pakistan. Arch Clin Infect Dis. 2014; 9(3): e17275.
11. Akhtar N, Bilal M, Rizwan M, Khan MA, Khan A. Genotypes of Hepatitis C Virus in Relapsed and Non-respondent Patients and their Response to Anti-Viral Therapy in District Mardan, Khyber Pakhtunkhawa, Pakistan. Asian Pac J Cancer Prev. 2014; 16(3):1037-1040
12. Zeeshan, M., & Shah, M. Chemiluminescent microparticle immunoassay-based detection on blood donor and incidence of HCV virus in district Mardan Pakistan.2019
13. Zeeshan, M., Anwar, F., Shah, I. A., & Shah, M. Prevalence of HBV, HCV, HIV and syphilis in blood donor at Mardan Medical complex, Khyber Pakhtunkhwa, Pakistan.2018
14. Anwar, F., Khan, M., Salman, M., Ahmad, S., Ullah, F., Khan, J., ... & Abbas, M. Seroprevalence of hepatitis B virus in human population of district Buner Khyber Pakhtunkhwa Pakistan. *Clinical Epidemiology and Global Health*. 2021*10*, 100688.
15. Umar M, tul Bushra H, Ahmad M, Ahmad M, Khurram M, Usman S, Arif M, Adam T, Minhas Z, Arif A, Naeem A. Hepatitis C in Pakistan: a review of available data. Hepatitis monthly. 2010;10(3):205.
16. Waheed Y, Shafi T, Safi SZ, Qadri I. Hepatitis C virus in Pakistan: a systematic review of prevalence, genotypes and risk factors. World journal of gastroenterology: WJG. 2009 Dec 7;15(45):5647.
17. Qureshi, H., Bile, K. M., Jooma, R., Alam, S. E., &Afrid, H. U. R. Prevalence of hepatitis B and C viral infections in Pakistan: findings of a national survey appealing for effective prevention and control measures. *EMHJ-Eastern Mediterranean Health Journal. 2010 16 (Supp.), 15-23, 2010.*
18. Anwar, F., Tayyab, M., Salman, M., Abdullah, Din, M., Khan, J., &Haq, I. Dengue outbreak 2018 in district Shangla KPK; clinical features and laboratory markers of dengue virus infection. *Future Virology*.2020 *15*(10), 693-699.
19. Jamil, Z., Waheed, Y., Malik, M., & Durrani, A. A. Effect of Sofosbuvir plus Ribavirin therapy on hepatitis C patients in Pakistan: a retrospective study. *PeerJ*. 2018 *6*, e4853.
20. Shah, I. A., Anwar, F., Haq, I. U., Anwar, Y., Aizaz, M., & Ullah, N. HBV burden on population, a comparative study between two districts Mardan and Charsadda of KPK, Pakistan. *International Journal of Contemporary Research and Review*.2018 *9*(09), 20269-20274.