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Prevalence of Differential HCV Genotypes and Their Treatment Regimen

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A B S T R A C T

Background: Hepatitis C virus (HCV) is a blood-borne virus that can cause chronic hepatitis, a life-threatening liver disease. HCV belong to the flavivirus family ith single-stranded RNA genome and has a 9.6-kb genome. HCV infects both humans and other mammals and can spread through blood or blood products. Hepatitis C virus can infect person of all ages, but hepatitis C virus found high incidence rate in adults.

Objective: The current study was intended to determine the prevalence of differential HCV genotype and their treatment regimen.

Methodology: The present study was carried out in Rehman medical institute (RMI) at department of Molecular diagnostic. The Hepatitis C Virus Genotyping (LINEAR ARRAY) Test was used for HCV Genotyping.

Results: The result showed that genotype 3 of HCV (1-6) was the most prevailing while genotype 1, genotype 1&3, genotype 1&4 is less common. The mix genotyping was recoded in 14 patients in which 07 is genotype 1&3 and 07 patients was recoded genotype 1&4 while genotype 3 was recoded.

Conclusion: Current study concludes that HCV genotype 3 was highly prevalent in Peshawar region of Pakistan while genotype 1, and mix genotype 1&3, mix genotype 3&4 is less common and the age group of majority of the patients were within 36 to 65 year and for the treatment of HCV the SOVALDI (Sofosbuvir) was the drug of choice as compared to available medication/treatment regimen n 47 patients.

Key words: HCV, Genotype, Prevalence, KPK, RMI, Pakistan.

Introduction

Hepatitis C virus is a blood borne pathogen and is transmitted mainly through parenteral route. The major mode of transmission is through blood and blood products.[1] The other mode of transmission is through sharing of needles, sexual contact and breast-feeding or at time of childbirth and from mother to child during pregnancy.[1] The incubation period of hepatitis C virus is usually between 2 -24 weeks. The clinical manifestations of hepatitis C virus infection include fatigue, malaise, anorexia, vomiting, nausea, dark urine, abdominal pain, clay-coloured stools, jaundice and hepatomegaly. The disease course of hepatitis C virus is variable and can range from asymptomatic infection to fulminant hepatic failure.[2] The asymptomatic infection can progress to chronic hepatitis C which can further lead to hepatocellular carcinoma and cirrhosis. There is no availability of vaccine against hepatitis C virus and the only way to prevent the infection is by avoiding exposure to the virus. The hepatitis C virus infection treatment includes interferon-based therapy and direct acting antiviral therapy. Hepatitis C virus is composed of two viral glycoprotein, E1 and E2 envelope protein and icosahedral capsid which have positive sense single standard RNA genome.[4] While genome of hepatitis C virus partially put into 10 proteins in which 4 are structural protein (core, E1, E2 and p7) and 6 are non-structural protein (NS4B NS3, NS2, NS4A, , NS5B and NS5A).[5] Viral hepatitis is a systemic disease mostly involve liver .[6] In human hepatitis C virus is prolong and acute hepatic inflammation. Hepatitis C virus is subjected to prolong hepatitis, hepatocellular carcinoma and liver cirrhosis.[2] Hepatocytes and

B-lymphocytes of human cell are mostly influenced by hepatitis C virus.[7] The duration of hepatitis C virus is infected 15-160 days while average is 50 days.6 Hepatitis C virus can involve person of all ages but majority of hepatitis C virus are found in a high rate in adult.[8]

Hepatitis C virus About 170 million people are chronically affects throughout the world.[11] North America is about 1% of this population.[7] High rate of infection has been found in Pakistan (4.8%), china (3.2%), Egypt (22%) reported by world health organization (WHO).[12] While in the following countries its rate are Afghanistan (1.1%), Myanmar (2.5%), Nepal (1.0%) Iran (0.87%) and India (0.66%).[13]

The common subtypes of HCV are 1a and 1b in Europe and America but subtype 1b are also found in Japanese population while subtype 2a and 2b are common in Europe, Japan and North America. Genotype 3 is arised from Asia, genotype 4 is most common in the Middle East and North African countries and genotype 5 and 6 are common in Hong Kong and North Africa. In Vietnamese genotype 7,8 and 9 are observed while genotypes 10 and 11 are commonly found in Indonesia.[7] Each one is different nucleotide at level by 31-33% and more several subtypes of ratings.[19] Relationship has been recommended among HCV genotype, serum HCV RNA levels and subtype.[20]

Direct acting antiviral treatment used to treat HCV infection. The infected individuals are unaware about the onset of this disease because of the asymptomatic nature of the disease, treatment for those who are diagnosed remains low in many hospital settings.[21] The standard therapy for hepatitis C virus is IFN-α plus ribavirin with 50% success rate.[22] The most important factor for hepatitis C virus treatment is genotyping. Every genotype differ in their reaction to interferon and ribavirin treatment while with combination of peg- interferon and ribavirin genotype 1 continue their response with success rate of 40% to 45% as compared to genotype 2 and 3 rate which will be 80%.[15] Genotype 1 treatment is more difficult as compared to genotype 2 and 3. Genotype 1 is caused severe liver disease than other type of genotypes.[23] The required time period for treatment of genotype 2 and 3 is 24 weeks while 48 weeks for treatment of genotype 1.[11] New treatment for hepatitis C virus is more impressive, short duration and less toxic than interferon-based therapies.[24] Some patients are not willing for interferon therapy for a variety of reasons, including to hate from injections and concern about adverse events associated with treatment.[25] Treatment of hepatitis C virus should be given by hepatologists, gastroenterologists or infectious disease experts.[26]

PCR was recommended as a gold standard method to detect Hepatitis C virus RNA and for monitoring the treatment of Hepatitis C virus.[16] Present study was conducted to check the prevalence of HCV genotype.

Methodology

The study was conducted in Rehman Medical Institute (RMI) at department of Molecular diagnostic. Total 64 samples of plasma were collected from hepatitis C virus infected patients for the analysis of HCV genotyping from patients visiting RMI clinics during year of 2013 to October 2016. In which 32 were male and 32 were female, from different cites of KPK. For this study, the approval from ethical committee was not required as samples of HCV patient were collected for clinical diagnoses purpose from different clinics of RMI. All of HCV patient sample were confirmed positive for HCV.

Specimen preparation for HCV genotyping were done with following chemicals which contain lysis solution Tris- HCl buffer, Guanidine thiocyanate HCV diluent and internal control(IC) as per protocols given by manufactures. Amplification of HCV genotype was done by amplification kit (AMPLICOR) By (Roche Molecular System Germany) following chemicals were used in amplification, which contain master mix solution Bicine buffer, DNA polymerase Potassium acetate, UTR HCV primer, Uracil-N-enzyme and sodium azide.

Detection of HCV genotypes were done by using LINEAR ARRAY detection kit contain Denaturing solution sodium hydroxide, sodium laurayl sulfate, sodium phosphate solution, sodium chloride, Streptavidin-Horseradish Peroxidase, citrate solution Tetramethylebenzidine, and Dimethyleformamide all procedure was done as per protocols given by manufactures.

Genotyping were done by using LINEAR ARRAY HCV Genotyping test strip containing 9 HCV specific genotype on a nylon strip coated with DNA probe and comparing bands by using reference card. HCV Genotyping was achieved with using Roche linear array Germany kit for specimen preparation, target amplification, and LINEAR ARRAY for detection. This is in vitro test used for the identification of Genotypes of HCV infected patients from GT1-GT6.

HCV RNA was extracted by using the specimen preparation kit according to the manufacturing protocol. HCV RNA was used as a template to generate cDNA by reverse transcription. The double stranded cDNA was then use to amplify the target by polymerase chain reaction (PCR). Detection of the amplicons for the HCV genotypes was confirmed by using LINEAR ARRAY detection kit by using the

HCV amplicons; oligonucleotide labelled probes hybridized with amplicons to which are specific to the target site; and colorimetric method was used for the detection of the probe-bound amplicons. On the strip, amplicon labeled with Biotin was binded to the different sites. Streptavidin-Horseradish Peroxidase was added which binds to the probe labelled with biotin after the step of hybridization and several washing steps. Then again washing and treatment with the substrate (3, 3', 5, 5'-tetramethylbenzidine and hydrogen peroxide) was done. As a result, blue color on strip appeared where hybridization has already been done. Experimental results were confirmed by reading the strips visually and by comparing the genotype patterns of reference card with blue bands.

Results

Distribution of HCV genotype on the basis of conventional PCR method 64 patients were confirm chronic hepatitis C virus and analyzed for Hepatitis C virus genotyping. Out of 64 positive patients 32 were male and 32 were female. By using the HCV Genotyping (LINEAR ARRAY) Test on the basis of HCV viral load we processed all the 64 sample.

Table I: Distribution of HCV genotype in studied Patients.

Genotype	N	Male	Female	Total
Genotype 1	01	00	01	01
Genotype 3	47	24	23	47
Mixed Genotype				
Genotype 1&3	07	05	02	07
Genotype 3&4	07	01	06	07

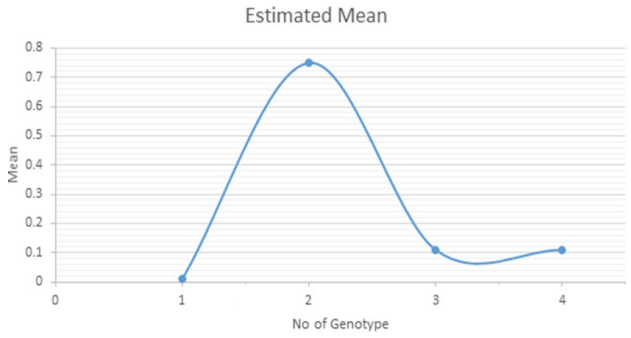

Figure 1. Estimated Mean of Genotype.

Discussion

Hepatitis C virus is a blood-born virus and belongs to the family of Flaviviridae and genus Hepacivirus. HCV has been divided into six major genotype and several of subtypes. The prevalence of HCV genotype is different between country and regions. The major region which is more affected by Hepatitis C virus is Africa, Central and East Asia.[10] In Asia genotype 3 is most common while other types of genotype 1a and 1b are found in in Europe and America but sub type 1b are also found in Japanese population While 2a and 2b are common in Europe, North America and Japan, south Africa genotype 5, Hong Kong genotype 6, Vietnam genotype 7, 8, 9 and Indonesia genotype 10, 11 is most common.[7] According to WHO 170 million people of the world population is affected from Hepatitis C virus[11], and about 3-4 million people are newly infected per year.[15] The estimated deaths per year was 53000 due to HCV infection.[14] Here in Pakistan genotype 3 and subtype 3a is most common. A study by Gul et al[18] identified that genotype 3a is most abundant in Peshawar region while other study by Mehmood et al [15] identified that genotype 3 is most common. The study by Ali et al [17] also identified that genotype 3a is most common while study at Bahawalpur[27] and Karachi [28] also shows that genotype 3 is common. It means that genotype 3 is most common in this region of the world. Our study results in line with these studies as genotype 3 is common in this region. In the present study all the sample were collected for diagnosis purpose from different Clinics of Rehman Medical Institute (RMI) and analyzed at department of Molecular diagnostic. All the patients belonged to different cities and district of Khyber Pakhtunkhwa (KPK). A total 64 samples were collected for HCV genotyping as all the sample were confirm positive for HCV. On the basis of LINER ARRAY Genotyping test for the detection of different HCV genotyping were done and getting result in this result two patients genotype were not detected while the most common genotype was 3 and genotype 1, genotype 1&3, genotype 3&4 is less common .So it means that genotype 3 is most common in this region of the world and after this in this study we collected the history from infected patient So majority of patients have used SOVALDI (Sofosbuvir) up to six months and after this treatment they were reported negative for HCV PCR.

Conclusion

This study concludes that genotype 3 of HCV is highly prevalent in circulation in this region of the world while genotype 1, and mix genotype 1&3, mix genotype 3 & 4 is less common, and the majority of the patients were within the age of 36 to 65 year and for the treatment of HCV the SOVALDI (Sofosbuvir) is the drug of choice as compared to available medication/treatment regimen.

References

1. District Laboratory Practice in Tropical Countries Part II Monica Cheesbrough. second Edi. Cambridg University Press; 2012. 250-253 p.
2. Rehman HU, Waqar M, Wasim M, Khan AU, Noor AA, Ahmad T, et al. Molecular detection of the Hepatitis C virus genotypes circulating among both sexes of Khyber Pakhtunkhwa , Pakistan. 2014;5(3):51-5. https://doi.org/10.3126/ajms.v5i3.9449

3. Popescu C-I, Riva L, Vlaicu O, Farhat R, Rouillé Y, Dubuisson J. Hepatitis C virus life cycle and lipid metabolism. Biology. 2014;3(4):892-921. https://doi.org/10.3390/biology3040892
4. Goossens N, Cl S, Negro F. Handbook of Hepatitis C Handbook of Hepatitis C [Internet]. Springer International Publishing Switzerland; 2016. 13-20 p. Available from: http://www.springer.com https://doi.org/10.1007/978-3-319-28053-0
5. Kanda T, Yasui S, Nakamura M, Suzuki E, Arai M, Haga Y, et al. Daclatasvir plus asunaprevir treatment for real-world HCV genotype 1-infected patients in Japan. International Journal of Medical Sciences. 2016;13(6):418-23. https://doi.org/10.7150/ijms.15519
6. Jawetz, Melnick & AMM. Medical Microbiology. 26th ed. McGraw Hill; 2013. 511-512 p.
7. Inamullah, Idrees M, Ahmed H, Sajid-ul-ghafoor, Ali M, Ali L, et al. Hepatitis C virus genotypes circulating in district Swat of Khyber Pakhtoonkhaw, Pakistan. Virology journal. 2011;8:16. https://doi.org/10.1186/1743-422X-8-16
8. Alter MJ. Epidemiology of hepatitis C virus infection. World Journal of Gastroenterology. 2007;13(17):2436-41. https://doi.org/10.3748/wjg.v13.i17.2436
9. Raja NS, Janjua KA. Epidemiology of hepatitis C virus infection in Pakistan. 2008;4-8.
10. WHO feact sheet 2016 [Internet]. WHO; 2016. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/
11. Khan TM, Mehr MT, Ullah H, Khan H, Iman NU, Complex HM, et al. Frequency of Hepatitis C Virus Genotypes in the North of Pakistan. Gomal Journal of Medical Sciences. 2014;12(2):5-8.
12. Bhatia V, Pandey M, Shashank M, Tidke P, Jha N, Dutt S. of Recent Scientific RESEARCH ARTICLE HCV GENOTYPES DISTRIBUTION PATTERN & ITS ASSOCIATION WITH VIRAL LOAD IN INDIA. 2013;4:1682-4.
13. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: a systemic review. Virology journal. 2011;8(1):433. https://doi.org/10.1186/1743-422X-8-433
14. Afridi S, Naeem M, Hussain A, Kakar N, Babar ME, Ahmad J. Prevalence of hepatitis C virus (HCV) genotypes in Balochistan. Vol. 36, Molecular Biology Reports. 2009. 1511-1514 p. https://doi.org/10.1007/s11033-008-9342-0
15. Mahmood K, Mohammad N, Abidullah. Genotype variation of hepatitis C virus in District Buner Swat. Journal Ayub Medical Collage Abbottabad. 2011;23(4):18-21.
16. Cheng PS, Yam WC. Evaluation and comparison of genotyping assays for molecular epidemiological study of HCV in Hong Kong. Jornal of Hong Kong Institute of Medical Laboratory Scicnce. 2007;11(2007).
17. Ali A, Ahmed H, Idrees M. Molecular epidemiology of Hepatitis C virus genotypes in Khyber Pakhtoonkhaw of Pakistan. Virology journal. 2010;7(1):203. https://doi.org/10.1186/1743-422X-7-203
18. Gul A, Zahid N, Ahmed J, Zahir F, Khan IA, Ali I. Molecular characterization of Hepatitis C virus 3a in Peshawar. BMC Infectious Diseases. 2016;16(1):163. https://doi.org/10.1186/s12879-016-1488-0
19. Treso B, Takacs M, Dencs a, Dudas M, Par a, Rusvai E. Molecular epidemiology of hepatitis C virus genotypes and subtypes among injecting drug users in Hungary. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2013;18(47):1-6. https://doi.org/10.2807/1560-7917.ES2013.18.47.20639
20. Chakravarti A, Dogra G, Verma V, Srivastava AP. Distribution pattern of HCV genotypes & its association with viral load. Indian Journal of Medical Research. 2011;133(3):326-31.
21. WHO. Guidelines for the Screening, Care and Treatment of Persons With Hepatitis C Infection [Internet]. 2016. Available from: http://apps.who.int/iris/bitstream/10665/205035/1/9789241549615_eng.pdf
22. Bashir1 Q, Rashid A, 2, Naveed AK, 2, Khan RSA, et al. Interferon Signalling Pathway. KMUJ. 2013;5(3):123-7.
23. LINEAR ARRAY Hepatitis C Virus Genotyping Test. 2013.
24. Howes N, Lattimore S, Irving WL, Thomson BJ. Clinical Care Pathways for Patients With Hepatitis C: Reducing Critical Barriers to Effective Treatment. Open forum infectious diseases. 2016;3(1):ofv218. https://doi.org/10.1093/ofid/ofv218
25. Jacobson IM, Gordon SC, Kowdley K V., Yoshida EM, Rodriguez-Torres M, Sulkowski MS, et al. Sofosbuvir for Hepatitis C Genotype 2 or 3 in Patients without Treatment Options. New England Journal of Medicine. 2013;368(2o):130423030016000. https://doi.org/10.1056/NEJMoa1214854
26. Improving access to , and completion of , hepatitis C treatment [Internet]. Public Health England. Public Health England; 2015. Available from: http://www.nta.nhs.uk/uploads/turning-evidence-into-practice-improving-access-to-hepatitis-c-treatment.pdf
27. Masroor A. Qazi, M. Fayyaz, G. M.D. Chaudhary A jamil, A. H. Malik Al gardezi and MH bukhari. Hepatitis C virus genotypes in Bahawalpure. E:/Biomedica. 2006;22(1):95-101.
28. Masood N. Distribution Frequency of Hepatitis C Virus Genotypes in Patients Attending Liaquat. Journal of Postgraduate Medical Institute. 2014;28(4):367-71.