ORIGINAL ARTICLE

Determination of Ankyrin Repeats Domain (ANK) of RNASE L Gene in Hepatitis C Patients and its effects on Viral Load

Anum Liaquat Ali, Syed M. Zahid Azam, Sikandar Ali, Saba Kamil
Dow University of Health Sciences, Karachi, Pakistan.

ABSTRACT

Background: Hepatitis C virus (HCV) is one of the major global causes of death. Different types of gene are involved as Ankyrin repeat domains of RNASE L gene. It performs a significant role in antiviral response, regulated by interferon, and involved in cleavage of RNA. Therefore, aim of this study was to identify Ankyrin repeat domain expression in Hepatitis C positive patients and correlate it with viral load of Hepatitis C.

Methods: In this study, a total of 80 HCV positive patient's whole blood samples were investigated. RNA was extracted from plasma followed by Real Time PCR for quantization of HCV viral load and genotypic analyses. DNA was also extracted from these samples followed by PCR amplification of Ankyrin repeat domain of RNASE L gene. Data was analyzed using SPSS

Results: All of the patients (n=80) included in study had HCV infection. Mean age of patients was 50.86 ±14.84 years. Among them, 48(63.8%) were males and 32 (36.1%) were females. Majority of patients were males and belonged to age group 58-73 years age. All HCV infected individuals 36 (45%) had HCV genotype 3 and had viral loads mean range 837404.21 ±1302. Therefore, Ankyrin repeats domain of RNASE L gene expression was high in HCV patients sample with viral load of 17.00±15.1.

Conclusion: Ankyrin repeat domain expression was observed in Hepatitis C patients and its significant correlation with viral load of Hepatitis C. Ankyrin repeat domain of RNASE L gene in conjunction with therapeutic intervention are required for establishing better strategies for controlling HCV infection.

Keywords: Hepatitis C Virus; Ankyrin Repeat; Ribonuclease L; Polymerase Chain Reaction (PCR).

Corresponding Author:
Dr. Anum Liaquat Ali
Dow University of Health Sciences,
Karachi, Pakistan.
Email: anum.mona@gmail.com
doi.org/10.36283/PJMD9-3/002

INTRODUCTION

Hepatitis C virus (HCV) is one of the major problems that are globally causing morbidity and death. The current estimates show increase in its prevalence 2.8% above the last decade, consequently leading to more than 185 million infected people worldwide[1,2]. HCV chronic infection is frequently linked with progress in cirrhosis of liver, liver failure, hepatocellular cancer, and death[3]. Antiretroviral active therapy has been possibly reduce the amount of HCV infection in worldwide and decreased the mortality and transmission over the last 20 years[4-6].

An excellent understanding of HCV infections requires increase strategies to stop new infections. There are so many risk factors involved in its transmission like needle injury, blood born disease etc[7, 8]. About 80% of cases are associated with chronic viral infections causing initiation of liver cirrhosis as in Hepatitis B virus (HBV)[9]. Many cellular protected reaction in viral infection individual by natural killer (NK) cells or T lymphocytes, cytokines, chemokines and interferons (IFNs), highlighted antiviral response. In addition to immune system Virus response, there is also up-regulation of some intrinsic gene like 2-5` Oligoadenylate Syntheta-

se (OAS) and ultimately causes the apoptosis of viral infected cells[10] Different types of gene mutations are also involved in HCV infection. Ankyrin repeat domains (ANK) have a significant role in antiviral response causing regulation of interferon and involved in the cleavage of viral RNA[11]. Therefore, aim of this study was to identify expression analysis of Ankyrin repeat domains in hepatitis C patients and to correlate HCV Viral load with expressions of Ankyrin repeat domains.

METHODS

This research study was conducted at section of molecular pathology, Dow Diagnostics Reference and Research laboratory (DDRRL) and National Institute of Liver and Gastro Intestinal Diseases (NILGID), Dow University of Health Sciences, Karachi, Pakistan. Blood samples were collected from patients. Before receiving the blood samples, consent forms were filled and all patients were informed in details regarding the research. This research study was conducted afterward receiving approval from IRB (Institutional Review Board) and BASR (Board of Advance Studies and Research). A total sample of 80 individuals having diagnosed HCV patients, the collected blood in Yellow gel tube was centrifuged. Plasma was used for the experiment. RNA viral extraction kit QIAamp RNA Viral Mini Kit (Germany, QIAGEN) was employed for RNA extraction from samples according to the manufacturer instructions. Briefly, RNA purification process was carried out using QIA amp spin columns in normal micro centrifuge tube.

The samples were loaded with buffer lysate on to spin column QIA amp, RNA was adsorbed on QIA amp membrane of silica – gel during a short centrifugation. The RNA bound to membrane QIA amp was washed in two centrifugation steps. Wash situations ensured complete subtraction of any remaining contaminants affecting without the RNA binding. Purified RNA was eluted from the spin column QIA amp in a solution concentrated form AE buffer and it was kept at – 80°C. RNA amount: RNA sample purified of 1µl was watered down with deionized water (H20) 100µl measured through spectrophotometer at with wave length 260nm with 280 nm inside a cuvette quartz. Then was make complimentary Cdna M-MuLV RT enzyme was used to make the cDNA. Subsequent procedure was developed to build the cDNA[12]. Product was stored at -70°C and for HCV viral was performing real time PCR. The quantitative Real Time PCR was done for the quantitation of HCV viral load by using the commercially available Artus HCV RG RT-PCR Kit (Qiagen, Germany) according to the manufacturer's instructions the cycling conditions and reaction mixture of PCR.

HCV genotyping was performed by using a commercially available Abbott Real Time HCV Genotype II Kit (Abbott, USA) on an automated nucleic acid extractor and Real Time-PCR system m2000 Sp (Abbott, USA) according to the manufactures instruction. This assay was based on Real Time PCR which targets the 5` UTR and NS5B region genome of HCV to detect the all six HCV genotype. Blood sample was used for DNA extraction, DNA extracted by using DNA QIAamp mini Kit according to manufactures protocol. Amplification of ANK domain of RNASE L to determine the quality and quantity of purified DNA. PCR reaction was performed in order to ascertain beta-globin gene amplification by using already published primers followed by the amplification of ANK domain. All amplified products of PCR reaction were analyzed using gel-electrophoresis method. 2% agarose gel was prepared with ethidium bromide with the final concentration of about 0.2-0.5µg/mL and results were noted under the light UV also photographed by documentation system. Data was analyzed using SPSS. Mean and standard deviation was taken for numeric data frequency and proportion were assessed for categorical variables.

RESULTS

In this study out of 80 patients 36 (45%) had HCV infection. Among them, 23 (63.8%) were males and 13 (36.1%) were females Mean age of patients was 50.86 ±14.84 years. Among them, 48(63.8%) were males and 32 (36.1%) were females. Quantification of HCV viral load in 80 HCV and positive samples showed 40 (47.3%) had viral load 22500-44720IU/ml, 30 (39.4%) had viral load 44820-66940IU/ml and 6 (7.8%) had viral load 66950-89,160IU/ml shown in Table 1. While the mean HCV viral load was 280,419IU/ml and Ankyrin repeat domain mean 17.000±15.187 Real Time PCR analyses by using a commercially available kit for HCV Genotype revealed that all of the 80 HCV positive samples have found to be infected with Genotype 3 viral load 180-89,160IU/ml).

Table 1: Hepatitis C viral loads with Ankyrin Repeat domain of RNASE L gene.

Groups	n (%)	Viral Load	Ankyrin Repeat Domain of RNASE L Gene
Group 1	4 (5.2%)	(180 - 22400IU/ml)	2
Group2	40(47.6%)	(22500 - 44720IU/ml)	32
Group3	30(39.4%)	(44820 - 66940IU/ml)	28
Group4	6(7.8%)	(66950 - 89,160IU/ml)	6

 doi.org/10.36283/PJMD9-3/002

For the analysis, ANK domain of the RNASE L Gene was amplified by PCR and describes well doing amplification of particular specific 302 bp band ANK domain of RNASE L gene. And 2% Agarose gel showing 302bp product obtained by PCR amplification of a region in ANK Domain of RNASE L gene, 02, 03, are amplified samples of ARD and LD is 50 base pairs DNA ladder shown Figure 1 and 2.

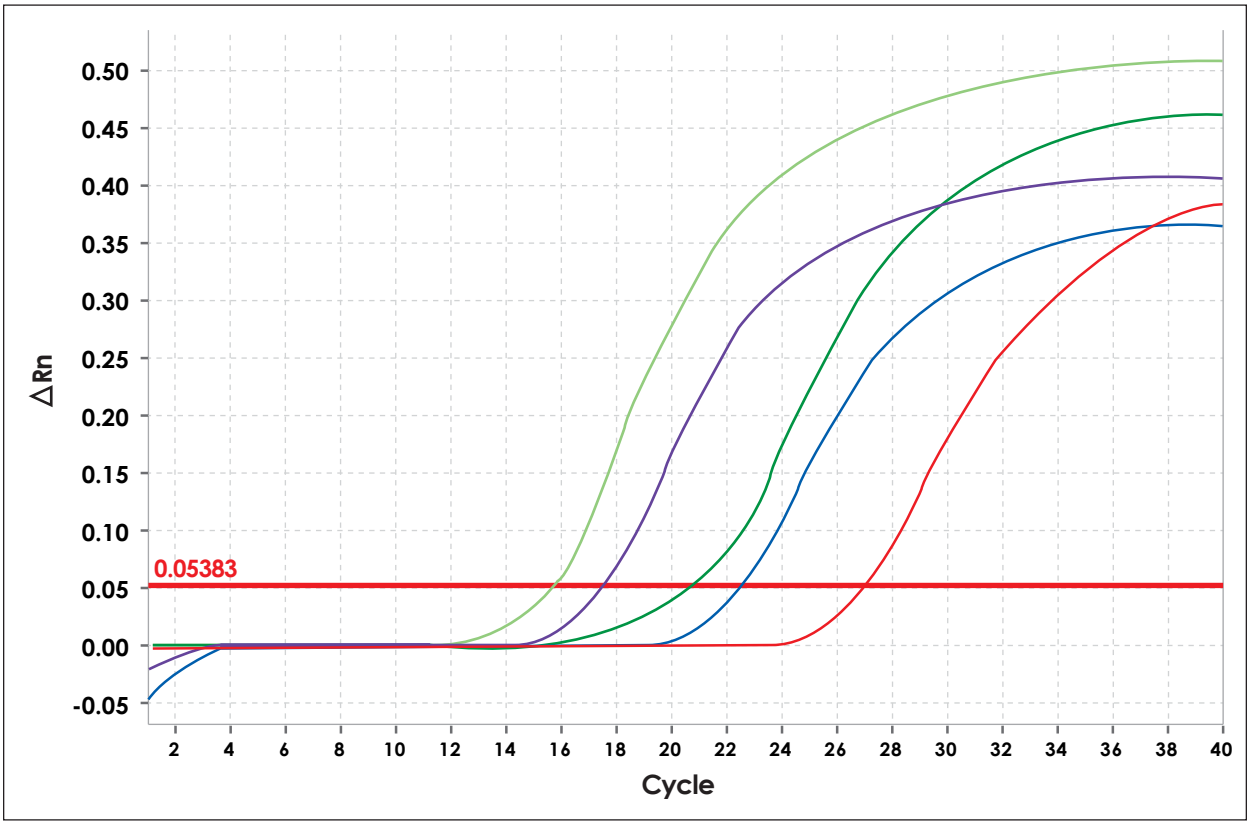


Figure 1: Real Time PCR for HCV viral load x-axis number of cycle, on y-axis 0.05383 is threshold value and these peaks are patients' samples.

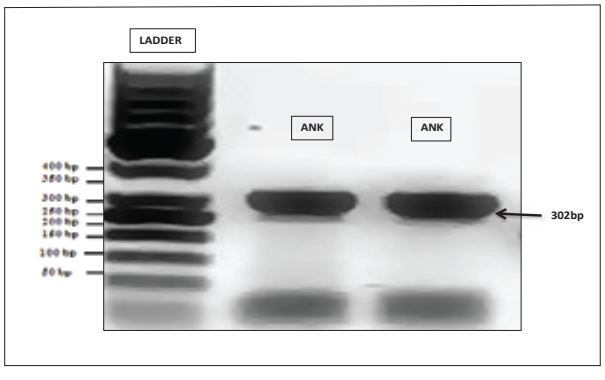


Figure 2: PCR-amplification of ANK domain of RNASE L gene.

DISCUSSION

HCV prevalence had seen in this study, jointly with the normally protracted natural history of HCV infection progression through viral load. It had already developed in literature that HCV show multiplicity and high viral load in developed liver disease among different countries. Countries with high frequency of HCV, peak incidence will have additional escalations in the HCV-related infections with hepatocellular carcinoma and cirrhosis in last two decades[13]. Above 184 million people, globally that have chronic HCV infection, mainly HCV old cases remained and HCV incidence increases with ever-increasing age awaiting the peak incidence at 55 to 64 years. In accordance to our study the 3 genotype is widespread in the South Asia in those people with chronic, HCV infection progressive hepatic fibrosis leading to the cirrhosis (15to35%) among age of 25 to 30 years) leading to mortality and morbidity[14].

The current Pakistan population is 193.2 million which is predominant of young individuals with progression of Hepatocellular carcinoma. However, Hepatitis C, is the greatest significant risk factor in the progression of hepatocellular carcinoma in the Pakistan[15,16]. Hepatitis C virus is the RNA virus, which causes develop of liver damage that might outcome in hepatocellular carcinoma and cirrhosis of liver. The most important risk factors is blood-borne infection by insecure injection of drug users and unsterile medical procedures. Diagnostic measures included HCV serum antibody testing, RNA HCV measurement, the viral genotype[17]. Genotype 3 of HCV significantly improved risk of the

hepatocellular carcinoma(HCC)while few patients of cirrhosis documented with adverse clinical outcomes[18,19]. Differences in the genetic factors risk not captured through our study, may well explain a number of experimental associations among HCV genotypes, cirrhosis and the HCC risk highlighted that hepatitis C is mainly common reason that causes HCC[19].

Different group of studies has researched about RNASE L with different risk factors. In breast (MMTV) and uterine cancer RNASE L gene has been detected and believed as major risk factor for disease progression. Defective RNASE L gene would be unable to clear out the viral infection that ultimately leads to carcinoma[17-19]. However, in this study ARD domain, which is crucial for Pakistani population is at higher risk for HCV association with cirrhosis and ultimately leading to HCC.

Human papilloma virus (HPV) and Epstein Barr virus (EBV) sequences from mouse mammary tumor, depicted RNASE L gene and RNASE domain activation, which degrade the cellular RNA, thus involved in viral clearance from the cell. Mutations in sequence cause the increase in HCV viral loads. Because RNASE L has antiviral activity[18] and if there is expression in ANK domain of RNASE L gene, high viral load was observed with mean range 837404.21 ±1302318. This was in accordance to our study, which showed high HCV viral load, and Ankyrin repeats domain of RNASE Gene expression with mean range 17.00±15.187. However, in the study we had only identified analysis of ANK domain of RNASEL gene expression in HCV patient who was also positive for HCV Genotype 3 with viral load 683,208 IU/ml. Expression had been observed in ARD domain of Hepatitis C patients. To the best of our knowledge, this was first research of its kind on HCV. Therefore, it is important to conduct more research on ANK domain of RNASE L analysis in HCV and its association with HCV viral load.

CONCLUSION

Ankyrin repeat domain expression was observed in Hepatitis C patients and its significant correlation with viral load of Hepatitis C. Ankyrin repeat domain of RNASE L gene in conjunction with therapeutic intervention are required for establishing better strategies for controlling HCV infection.

ACKNOWLEDGMENTS

We are especially thankful to the National Institute of Liver Disease (NILGID) and grateful to DDRL Dow University of Health Sciences Karachi.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ETHICS APPROVAL

The authors obtained the ethics approval from the Dow University of Health Sciences with the reference code of IRB-1038/DUHS dated on May 12, 2018.

PATIENT CONSENT

Proper written consents were obtained for the mentioned study.

AUTHORS' CONTRIBUTION

ALI involved in collection of HVC patients sample from, NILGID, DUHS. Also did the experiment, analysis, and compilation of the data. ZA clinically identified the HCV patients.

REFERENCES

1. Blach S, Zeuzem S, Manns M, Altraif I, Duberg AS, Muljono DH, *et al*. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2(3):161-76.
2. Bhatti H, Bakar A, Dar FS, Waheed A, Shafique K, Sultan F, *et al*. Hepatocellular carcinoma in Pakistan: national trends and global perspective. Gastroenterol Res Pract. 2016.
3. Ghouri YA, Mian I, Rowe JH. Review of hepatocellular carcinoma: Epidemiology, etiology, and carcinogenesis. J Carcinogen. 2017;16.
4. Kim HS, El-Serag HB. The epidemiology of hepatocellular carcinoma in the USA. Curr Gastroenterol Rep. 2019;21(4):17.
5. Kuo YH, Kee KM, Hsu NT, Wang JH, Hsiao CC, Chen Y, *et al*. Using AST-platelet ratio index and fibrosis 4 index for detecting chronic hepatitis C in a large-scale community screening. PloS one. 2019;14(10).
6. Makris S, Paulsen M, Johansson C. Type I interferons as regulators of lung inflammation. Front Immunol. 2017;8:259.
7. Jack K, Thomson B, Linsley P, Irving W. A survey of risk factors for Hepatitis C Virus infection and test uptake in an English male prison. InEASL Int Liver Congress. 2019.
8. McMahon BJ, Bruden D, Townshend-Bulson L, Simons B, Spradling P, Livingston S, *et al*. Infection with hepatitis C virus genotype 3 is an independent risk factor for end-stage liver disease, hepatocellular carcinoma, and liver-related death. Clin Gastroenterol Hepatol. 2017;15(3):431-7.
9. Li Y, Banerjee S, Wang Y, Goldstein SA, Dong B, Gaughan C, *et al*. Activation of RNase L is dependent on OAS3 expression during infection with diverse human viruses. Proc Natl Acad Sci. 2016;113(8):2241-6.
10. Palikša S, Alzbutas G, Skirgaila R. Decreased Km to dNTPs is an essential M-MuLV reverse transcriptase adoption required to perform efficient

cDNA synthesis in One-Step RT-PCR assay. Protein Eng Des Sel. 2018;31(3):79-89.
11. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol. 2013;10(9):553.
12. Thrift AP, El-Serag HB, Kanwal F. Global epidemiology and burden of HCV infection and HCV-related disease. Nat Rev Gastroenterol Hepatol. 2017;14(2):122.
13. Lok AS, Seeff LB, Morgan TR, Di Bisceglie AM, Sterling RK, Curto TM, *et al*. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroe-nterol. 2009;136(1):138-48.
14. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61(1):S45-S57.
15. Manns MP, Buti M, Gane E, Pawlotsky JM, Razavi H, Terrault N, *et al*. Hepatitis C virus infection. Nat Rev Dis Primers, 2017;3(1):1-9.
16. Pradat P, Trepo C. HCV: epidemiology, modes of transmission and prevention of spread. Best Pract Res Clin Gastroenterol. 2000;14(2):201-10.
17. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007;13(17):2436.
18. Madsen BE, Ramos EM, Boulard M, Duda K, Overgaard J, Nordsmark M, *et al*. Germline mutation in RNASEL predicts increased risk of head and neck, uterine cervix and breast cancer. PLoS One. 2008;3(6).
19. Farzan SF, Karagas MR, Christensen BC, Li Z, Kuriger JK, Nelson HH. RNASEL and MIR146A SNP-SNP interaction as a susceptibility factor for non-melanoma skin cancer. PloS one. 2014;9(4).