Clinical Case Reports International

Research Article
Published: 29 Apr, 2022

Comparison of HCV Genotyping Methodologies (Real-Time PCR and Conventional PCR)

*Bushra Uzair *, Sadia Abid, Asma Saleem and Kanwal Bibi*

Department of Bioinformatics and Biotechnology, International Islamic University, Pakistan

Abstract

A considerable amount of attention has been paid on hepatitis C virus infection due to its fatal consequences. Genotyping is an essential tool to predict the outcome of better treatment and control of this infection. 50 samples from patients with HCV infection within the ages from 20 to 65 years were collected. HCV Ab ELISA kit was used for the detection of virus in the samples which tested 43 patients HCV RNA positive. Maximum viral load detected in patients was 9.4×10^7 IU/mL and the minimum quantity of viral load was 1.2×10^5 IU/mL. The major mode of transmission of virus which caused hepatitis in patients included unsafe blood transfusion, use of unsterile needles and dental instrument. In our study 37 patients were detected with 3a genotype, 2 with 1a genotype and genotype of 4 patients was untypable. Further analysis of these patients' genotype by Real-Time PCR revealed that they were also infected by 3a genotype. Overall assessment showed that genotype 3a is the most prevalent genotype affecting 95% patients followed by 1a. Only males were infected by genotype 1a due to travelling to neighbor countries. Comparative analysis on genotyping methodologies suggests that Real-Time PCR has advantage over traditional PCR. Conventional PCR is less effective method because of its poor resolution, less sensitivity and risk of contamination. It is time consuming process and yield results at the end point of reaction. In contrast, Real-Time PCR has enhanced rapidity, reproducibility, sensitivity and the reduced risk of contamination. In addition, it can resolve 95% of HCV genotypes and can provide data at exponential growth phase of the reaction.

Keywords: Hepatitis C virus; HCV-RNA; Genotyping; Real–Time PCR

Introduction

Viral hepatitis is one of leading killers across the globe since 1989. Hepatitis C virus is a blood borne virus and mainly affects the function of liver causing acute or chronic liver diseases [1]. Patients developing cirrhosis have high risk of HCC (1% to 4% per year) prompting liver transplantation. HCV is considered second most common cause of viral hepatitis [2]. On the basis of Phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of Hepatitis C Virus (HCV) have been recognized [3]. Common subtypes that cause disease are 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, 6a.

WHO reported that globally 3.3% people are HCV infected and causes 700,000 deaths annually? The prevalence of HCV varies by region. HCV prevalence is lower (<2%) in regions of Western Europe, the Americas and Australia. Areas of African and the eastern Mediterranean have highest percentage of HCV prevalence [4]. Country with the highest prevalence of HCV in the world is Egypt, having more than 14% infected people [5,6]. Estimates show that prevalence in Asia is about 2% but it differs greatly between individual countries [7]. As Mongolia shows the highest HCV prevalence which is above 10%, Uzbekistan and Pakistan are next having 6% of total population infected with HCV [7]. Data on the HCV prevalence in Pakistan shows that it is highly endemic, infecting almost 6.8% population of the country. Most common HCV genotype is 3a which continues to infect almost 61.3% people in Pakistan. However, recently the frequency of subtype 2a has increased in few areas of Pakistan. Subtype 2a was most common in Khyber Pakhtunkhwa infecting 17.3% population and in Sindh provinces infecting 11.3% of the total population [8].

Incredible advancement has been made over the previous years in explaining the structure and function of the Hepatitis C Infection (HCV) proteins, a large portion of which are currently effectively being sought after as antiviral targets. Discovered in 1989 [9], HCV is a small enveloped virus with approximately 10 kb long plus-sense, single stranded RNA genome. It belongs to the *Hepacivirus* genera of *Flaviviridae* family [10]. Hepatitis C contains one long Open Reading Frame (ORF) which encodes for a non-functional polyprotein of approximately 3,000 to 3,010 amino

OPEN ACCESS

Correspondence:
Bushra Uzair, Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan,
E-mail: bushra.uzair@iiu.edu.pk

Received Date: *14 Mar 2022*
Accepted Date*: 26 Apr 2022*
Published Date*: 29 Apr 2022*

Citation:
Uzair B, Abid S, Saleem A, Bibi K. Comparison of HCV Genotyping Methodologies (Real-Time PCR and Conventional PCR). Clin Case Rep Int. 2022; 6: 1322.

Copyright *© 2022 Bushra Uzair. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

acids. This non-functional polyprotein is cleaved by signal peptidase, cellular and viral encoded proteases to yield at least 10 different viral protein products. Structural proteins which forms the viral particle, are encoded in the 5' N-terminal part of ORF and include C, E1, E2 and P7, whereas the non-structural proteins are encoded in the 3' part of the ORF in the order NS2, NS3, NS4A, NS4B, NS5A, NS5B [11]. HCV enters the cells through endocytosis after binding to particular receptors. The viral genome is translated into a precursor polyprotein subsequent to uncoating, and viral RNA is incorporated by a virus encoded polymerase complex. Newly synthesized viral particles are discharged into endoplasmic reticulum and are secreted from cell passing through Golgi apparatus [12]. Epidemiological studies revealed that the spread of HCV in Pakistan is through different modes of transmission which include blood transfusion, sexual activity, and intravenous drug abuse, unsafe use of needles, non-sterile dental instruments and hemodialysis [13-16]. HCV also spreads through non-sexual household contacts, ear piercing, tattoos, and use of community barber shops [17-19].

Medical and public health literature in detail on the prevalence of HCV in Pakistan has been reviewed by scientists for long period. In order to decrease burden of HCV in Pakistan awareness programs are required targeting rural areas. Different genotypes of HCV have specific drug sensitivity according to which the therapy is recommended [20].

There is currently no licensed vaccine for HCV, which is a worldwide health issue, recently infecting 3 to 4 million people annually. In present HCV vaccine approaches include peptide, plasmid DNA, recombinant proteins and vector-based vaccines. HCV treatment is based on pegylated-Interferon-a (IFNa) and the nucleoside analogue ribavirin, which is expensive, toxic, prolonged (24 to 48 weeks) and leads to a Sustained Virological Response (SVR) in only 50% to 60% of patients, depending on the infecting genotype [21].

The aim of our study was to evaluate and compare the performance features of two currently available HCV RNA quantification procedures including Real-time PCR based assays and conventional PCR based assays. Real-Time PCR methods are sensitive with lower limits of detection/quantification on the order of 10 to 15 HCV RNA International Units (IU)/ml, and are not prone to carryover contamination. It is done to identify the genotypes circulating in Pakistan and to determine the suitability of the HCV genomic region for routine determination of HCV genotypes. The Abbott Real-time HCV assay was used to evaluate the genotype and then it was compared to the conventional PCR method. The intrinsic and clinical performances of the recently developed Abbott m2000 real-time PCR system for HCV RNA quantification, which uses the automated Extractor m2000sp and the m2000rtdevice for automated real-time PCR amplification and detection of PCR products is examined.

Material and Methods

Serological diagnosis

In order to diagnose HCV infection, plan antiviral treatment programs and evaluate virological response to antiviral therapy, serological and virological tests play a key role. Serological diagnosis of HCV infection is based on two categories of laboratory tests, namely serologic assays detecting specific antibody to HCV (anti-HCV) (indirect tests) and, molecular assays that can detect, quantify, or characterize the components of HCV viral particles, such as HCV RNA and determine HCV genotype (direct tests) prior to sample collection diagnosis of patients was done. ELISA was performed to test positive antibodies to HCV. HCV-RNA detection in plasma was carried out by Qualitative RT-PCR (Abbott m2000rt) with lower limit of detection of 50 IU/ml.

Sample collection

In the present study 3cc blood was collected from 50 patients with detectable HCV RNA using disposable syringes. Blood samples were properly labeled with patient's name and Lab ID number for convenient analysis. Blood was transferred into EDTA coated vacutainer. In our study 43 patients out of 50 were tested positive for HCV, on which further diagnosis was performed.

Plasma separation

Samples were left at room temperature before centrifugation. The sample was centrifuged at 3000 rpm for 15 min and 750 ml of plasma was collected. Plasma was carefully stored at -80°C to avoid contamination until further use.

RNA extraction by Abbott m2000sp: The automated steps of the extraction system include sample and reagent pipetting, heating and incubation, magnetic capture and washing, and elution, all of which are optimized in ready-to-use protocols

1. Sample is prepared for the extraction and concentration of RNA molecules to help amplification of target region and removal of potential inhibitors from the sample.

2. The Abbott m2000sp instrument prepares samples for the Abbott Real-time HCV assay using the Abbott mSample Preparation System reagents.

3. The Abbott m2000sp uses magnetic particle technology to attach nucleic acids and particles are washed to remove unbound components from the sample.

4. The bound nucleic acids are eluted and transferred to a 96 deep-well plate. The nucleic acids are then prepared for amplification.

5. The IC (Internal Control) is placed through the complete sample preparation procedure along with the calibrators, controls, and specimens.

Amplification master mix:

1. The Abbott m2000sp instrument automatically assembles the amplification master mix (HCV Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent) and then transfers aliquots of the master mix to the Abbott 96-Well Optical Reaction Plate.

2. Nucleic acid samples are then transferred into the Abbott 96-Well Optical Reaction Plate by the Abbott m2000sp from the Abbott 96 Deep-Well Plate.

3. The plate is fixed by the user with an Abbott Optical Adhesive Cover and set into the Abbott m2000rt instrument for PCR amplification and fluorescence detection.

Amplification:

1. During the amplification reaction on the Abbott m2000rt, the target RNA is transformed to cDNA by the activity of reverse transcriptase of the thermostable rTth DNA polymerase.

2. HCV and IC reverse primers anneal to their particular

targets and are extended during a prolonged incubation period.

3. After incubation, the temperature of the reaction is raised above melting point of double stranded cDNA leading to denaturation step: RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

4. During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered.

5. Exponential amplification of the product is achieved through continual cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences.

6. Amplification of both targets (HCV and IC) takes place simultaneously in the same reaction.

Quantitation:

1. Quantitation of HCV RNA concentration of samples and controls requires a calibration curve.

2. Two assay calibrators are run in replicates of three to generate a calibration curve. The calibration curve slope and intercept are calculated from the assigned HCV RNA concentration and the median observed threshold cycle for each calibrator and are stored on the instrument.

3. The concentration of HCV RNA in specimens and controls is calculated from the stored calibration curve, and the results are automatically reported on the Abbott m2000rt workstation.

4. The Abbott Real-time HCV Negative Control, Low Positive Control, and High Positive Control must be included in each run to verify run validity. The Abbott m2000rt verifies that the controls are within the assigned ranges.

Determination of HCV viral load

HCV viral load was determined by using Abbott Real-time HCV genotype II amplification reagent on real time PCR instrument Abbott m2000sp.

Genotyping: Primer based genotyping by core region of HCV

cDNA synthesis:

- PCR water: 2 µL
- 5x RT buffer: 4 µL
- dNTPs 10mM: 1 µL
- RT enzyme: 1 µL
- Antisense Primer: 1 µL
- RNase inhibitor: 1 µL
- Total: 10 µL

10 µL RNA + 10 µL cDNA Master Mix = 20 µL

The extracted 10 µL RNA was added with 10 µL of cDNA Master Mix containing 4 µL of 5x RT buffer, 2 µL PCR water, 1 µL antisense primer, 1 µL dNTPs, 1 µL RNase inhibitor and 1 µL RT enzyme in eppendorf tubes and placed in Thermal Cycler.

Synthesis conditions are 42°C and 60 min.

The synthesized cDNA was stored at 4°C for short term storage or -20°C for long term storage.

Regular PCR: A 50 µL of Regular Master Mix containing 25 µL Dream Taq Master Mix, 1 µM Forward primer, 1 µM Reverse primer, 50 µL nuclease free water and 10 µL cDNA templates was prepared. The reaction mixture was placed in Thermal Cycler and HCV Genotype regular program was run.

- Dream Taq master mix: 25 µL
- Water to: 50 µL
- Forward primer: 0.1-1 µM
- Reverse primer: 0.1-1 µM
- cDNA: 10 µL
- Total: 50 µL

40 µL Regular Master Mix + 10 µL cDNA = 50 µL

Amplification cycles were shown in Figure 1.

Nested PCR: Nested PCR was performed in two separate mixtures. In Mixture I: 7 µL of Dream Taq Master Mix, 5 µL water, 1 µL each of S7, S2a, G1b, G2a, G2b, G3b primers and 2 µL Regular PCR product was used. For Mixture II: 7 µL of Dream Taq Master Mix, 5 µL water, 1 µL each of S7, G1a, G3a, G4, G5a, G6a primers and 2 µL Regular PCR product was utilized. PCR tubes were placed in Thermal Cycler and HCV-nested Program was run (Table 1).

Amplification conditions were shown in Figure 2.

Agarose gel electrophoresis

After PCR, the synthesized cDNAs were run on gel electrophoresis to detect HCV bands on gel

Preparation of gel: 2 gm of Agarose was mixed in 100 ml of 1X TBE buffer. The solution was heated in oven for 1 min 20 sec until homogenized. The solution was allowed to cool for 1 min. 2 to 3 drops of Ethidium Bromide were added to the solution.

Pouring of gel: The comb was placed in the gel caster and solution was gently poured into it. 100 ml of buffer solution was poured in electrophoretic chamber.

Solidifying: Solution poured in gel caster was left for 1 h to solidify into gel. The comb was removed and gel was transferred to electrophoretic chamber. PCR product was loaded in the wells of gel.

Gel run: The electrodes of electrophoretic chamber were connected and current of 92 volts was passed through the electrodes.

Gel documentation: Gel was removed from electrophoretic chamber and placed in Gel Doc BIO-RAD system for the detection of HCV genotypes through imaging of gel. DNA ladder of 100 bp was used to identify the bands formed on gel.

Results

Patient enrollment and selection

Forty-three patients were involved in the study and eight control groups of QCMD (Quality Control Molecular Diagnosis) were used for genotyping assay. Out of 43 patients, 19 were male and 24 were females. All the patients were tested positive for HCV antibodies by ELISA and had detectable HCV RNA in the serum. Most of the patients acquired HCV from Blood transfusion, non-sterile use of needles and dental instruments, while others mode of infection was

Table 1: Nested PCR was performed in two separate mixtures.

Mixture I		Mixture II	
Dream Taq Master mix	7 µL	Dream Taq Master mix	7 µL
Water	5 µL	Water	5 µL
S7	1 µL	S7	1 µL
S2a	1 µL	G1a	1 µL
G1b	1 µL	G3a	1 µL
G2a	1 µL	G4	1 µL
G2b	1 µL	G5a	1 µL
G3b	1 µL	G6a	1 µL
Regular PCR product	2 µL	Regular PCR product	2 µL
Total	20 µL	Total	20 µL

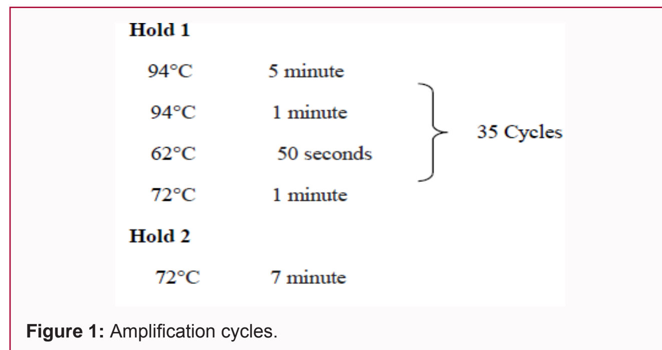

Figure 1: Amplification cycles.

Table 2: Gender Distribution of Hepatitis C patients according to age group.

Age Groups	No. of patients	Male			Female		
		1a	3a	Untypable	1a	3	Untypable
<25	3	-	2	-	-	1	-
25-35	9	-	3	1	-	5	-
>35-45	7	-	3	-	-	4	-
45-55	13	1	5	-	-	6	2
>55	10	1	3	-	-	5	1
Total	43	19 (42.2%)			24 (55.8%)		

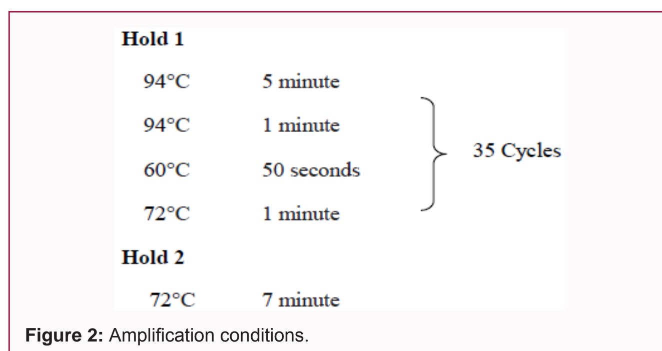

Figure 2: Amplification conditions.

unknown.

Regular PCR results

Gender distribution of HCV genotypes in patients according to age groups: Out of 19 male patients, 2 patients were diagnosed with 1a genotype, 16 patients were diagnosed with genotype 3a and 1 was HCV Indeterminate. 3 out of 24 female patients were untypable while remaining 21 were diagnosed with genotype 3a (Table 2).

Patients were divided into five age groups ranging from less than 25 to more than 55 years of age. Patients from age 45 to 55 years had highest percentage (23.25%) of HCV genotype 3a and 2.32% patients had genotype 1a, while 4.6% patients were untypable. In age group 25 years to 35 years 25% patients had genotype 3a and 2.32% patients were untypable. In age group >55 years 18.6% patients had genotype 3a, 2.32% patients had genotype 1a while 2.3% patients were untypable (Table 3, Figure 3).

Table 3: Distribution of age groups to study different genotypes among Patients.

Age	1a	3a	Untypable	Total
<25	0	3	0	3 (7%)
25-35	0	9	1	10 (23.2%)
>35-45	0	7	0	7 (16.3%)
45-55	1	10	2	13 (30.2%)
>55	1	8	1	10 (23.2%)
Total	2	37	4	40 (100%)

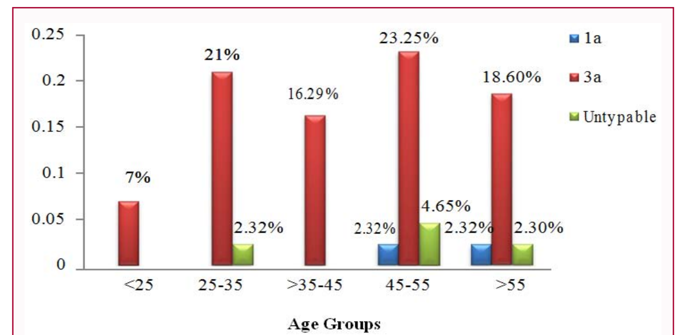

Figure 3: Graphical representation of hepatitis C genotype in different age groups.

Table 4: Genotype of Patients according to different areas.

Area	1a	3a	Untypable	Total
AJK	0	2	1	3 (7%)
Attock	0	1	0	1 (2.30%)
Chakwal	0	1	0	1 (2.30%)
Gujar Khan	0	1	0	1(2.30%)
Haripur	0	1	0	1 (2.3%)
Islamabad	1	17	2	20 (46.5%)
Mardan	0	1	0	1 (2.3%)
Murree	0	1	0	1 (2.3%)
Rawalpindi	1	12	1	14 (32.6%)
Total	2	37	4	43 (100%)

Genotyping in patients from different areas

Patients from nine different areas of Pakistan were involved in our study in which 39.53% patients from Islamabad had genotype 3a, 2.32% patients had genotype 1a and 4.65% patients were untypable. From Rawalpindi 27.9% patients had genotype 3a, 2.32% patients were detected with genotype 1a and 2.32% patients were untypable. 4.65% patients from AJK had genotype 3a and 2.32% patients were untypable. Patients from Attock, Chakwal, Gujar Khan, Haripur, Mardan and Murree had same genotype 3a (Table 4, Figure 4).

Real-Time PCR Statistical Analysis

Core region

Gender distribution of HCV genotype in patients according

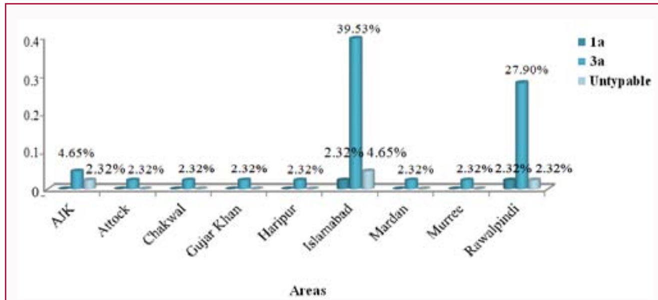

Figure 4: Graphical representation of genotyping in Patients from different areas.

Table 5: Gender distribution of Hepatitis C patients.

Gender	In-house		Total
	1a	3a	
Male	2	17	19 (44.2%)
Female	0	24	24 (55.8%)
Total	2	41	43 (100%)

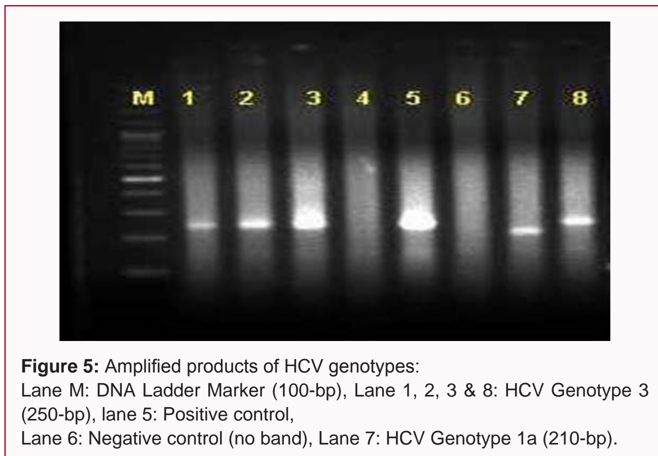

Figure 5: Amplified products of HCV genotypes:
Lane M: DNA Ladder Marker (100-bp), Lane 1, 2, 3 & 8: HCV Genotype 3 (250-bp), lane 5: Positive control,
Lane 6: Negative control (no band), Lane 7: HCV Genotype 1a (210-bp).

Table 6: Prevalence of HCV in different age groups.

Age	In-house		Total
	1a	3a	
<25	0	3	3 (6.97%)
25–35	0	10	10 (23.25%)
>35–45	0	7	7 (16.2%)
45–55	1	12	13 (30.23%)
>55	1	9	10 (23.25%)
Total	2	41	43

to age groups: Two male patients were diagnosed with 1a genotype while other 17 out of 19 had genotype 3a. Females were more than males in number and all had genotype 3a (Table 5, Figure 5).

Prevalence of HCV according to age groups

Patients were categorized into five age groups ranging from less than 25 to more than 55. Highest percentage (27.9%) of genotype 3a was observed in 45 to 55 years age group (Figure 6). In age group 25 to 35 years, 23.25% patients had genotype 3a while in age group >55 years, 20.93% patients had genotype 3a. Increase in percentages for the following age groups is due to the detection of untypable genotype by Real-time PCR as genotype 3a (Table 6, Figure 7).

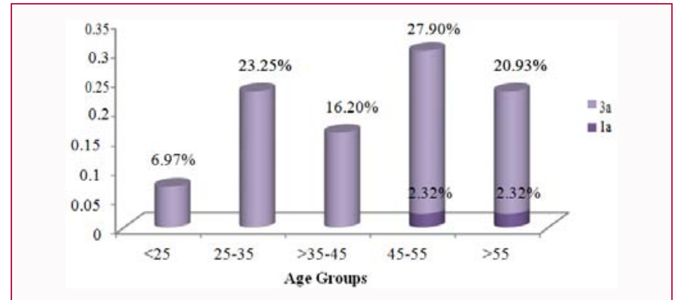

Figure 6: Graphical representation of genotypes among different age groups.

Table 7: Genotypes in patients from different regions of Pakistan.

Area	In-house		Total
	1a	3a	
AJK	0	3	3 (7%)
Attock	0	1	1 (2.3%)
Chakwal	0	1	1 (2.3%)
Gujar Khan	0	1	1 (2.3%)
Haripur	0	1	1 (2.3%)
Islamabad	1	19	20 (46.5%)
Mardan	0	1	1 (2.3%)
Murree	0	1	1 (2.3%)
Rawalpindi	1	13	14 (32.6%)
Total	2	41	43 (100%)

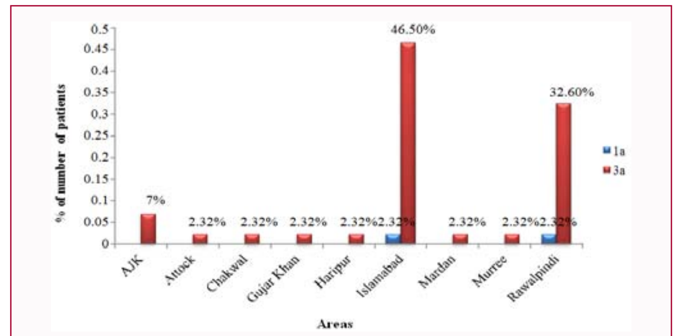

Figure 7: Graphical representation of genotypes in different regions of Pakistan.

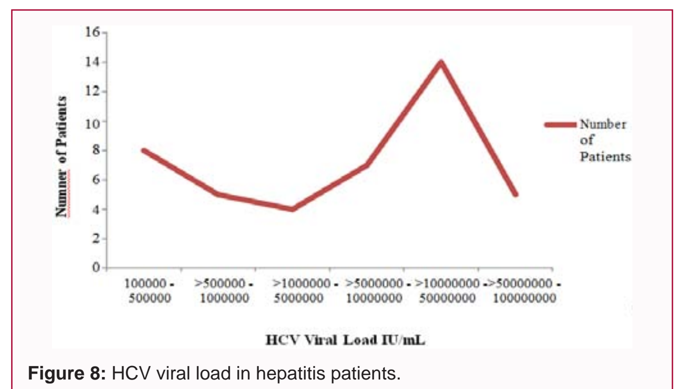

Figure 8: HCV viral load in hepatitis patients.

Genotyping of patients from different regions of Pakistan

Genotyping of patients from nine different areas was performed. 46.5% of patients from Islamabad, 32.6% patients from Rawalpindi and 7% patients from AJK had genotype 3a respectively. Shift in

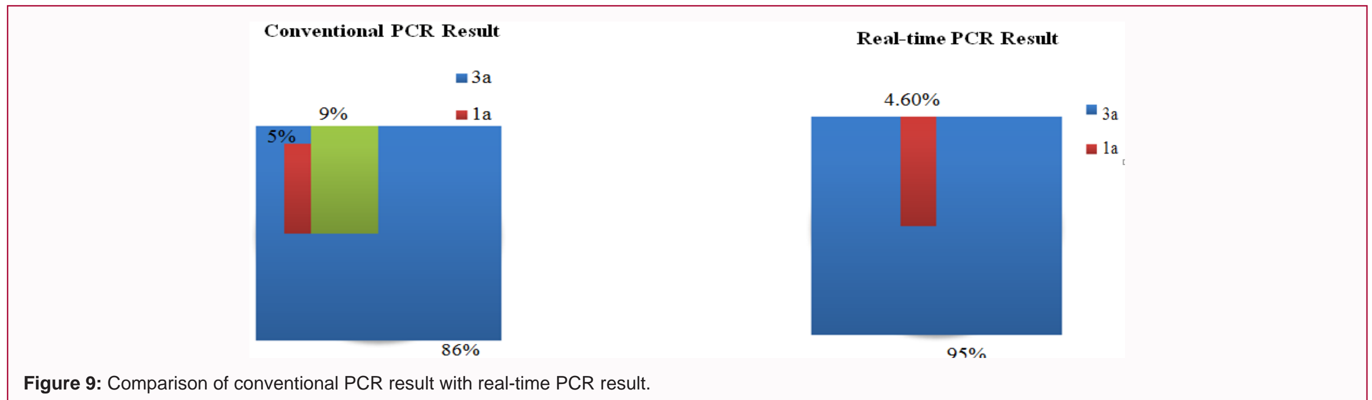

Figure 9: Comparison of conventional PCR result with real-time PCR result.

percentages from lower to higher in these areas as compared to conventional PCR results is due to detection of untypable genotype with genotype 3a by Real-time PCR.

HCV viral load

Patients were categorized according to viral load into six groups ranging from 100000 – 100000000. Maximum viral load detected in patients was 9.4×10^7 IU/mL and the minimum quantity of viral load was 1.2×10^5 IU/mL. Analysis of our data showed that greater number of patients were in the category of viral load ranging from >10000000 IU/mL to 50000000 IU/mL (Figure 8).

Comparison of conventional PCR and real-time PCR

Through statistical analysis it was observed that genotypes of patients that were identified as Untypable (9.3%) by conventional PCR were detected with genotype 3a by Real-time PCR. Percentage of genotype 3a patients (86%) detected through conventional PCR shifts to 95% when detected through Real-time PCR (Figure 9).

Discussion

Hepatitis C virus is one of the leading causes of death and disability worldwide and also getting endemic in many parts of Pakistan because very limited data on the genotyping of HCV has restricted the treatment of patients. In this study, we compared conventional PCR and Real–Time PCR genotyping methodologies of HCV to evaluate their efficiency to diagnose genotype of HCV patients.

In our study percentage of HCV genotype 3 was observed higher (95.3%) than genotype 1a (5%). It is proven in other studies done by [8], which claimed that 3a genotype is the major genotype in Pakistan followed by 1a genotype. Also in our study genotype 1a was second major genotype.

The predominance of genotype 3a in Pakistan is a good hope as it requires short duration of treatment and control of hepatitis C than genotype 1a [22]. The occurrence of HCV 3 genotype in our population confirmed the occurrence of HCV genotype in neighboring countries including China, Iran, Afghanistan and India. In the west, Baluchistan shares a long border with Iran where genotype 1a and 3a are most prevalent. It is reported that there is the high prevalence of genotype 3a and 1b in China [23]. So, it is quite possible that other genotypes and subtypes can be transferred from surrounding countries to Pakistan. In our study, only males were infected by HCV genotype 1a while all females were infected by genotype 3a. High percentage of genotype 1a in males is due to traveling in neighboring countries in search of jobs, trade and developing career opportunities [24]. Shifts in HCV genotype distribution needs to be noticed as it is an alarming signal to take major steps to reduce infections caused by other genotypes because genotype 1a and 4a are responsible for severe cirrhosis.

We compared the results of conventional PCR with Real-time PCR results. Those patients whose genotype was untypable were detected with 3a by Real-time PCR. This proved that Real-time PCR is accurate method that can detect each genotype infecting a patient. Real-time PCR in contrast to conventional PCR is rapid and reproducible method for the assessment of Hepatitis C virus load and genotype in serum samples. Quantitative PCR method such as regular or conventional PCR detects viral load and measures amplification product at the final phase of the reaction [25]. Similar study suggests that at the exponential phase of PCR the quantity of product is directly proportional to the initial number of template copies. As the reaction reaches plateau phase this correlation is lost. In contrast to regular PCR, Real Time PCR allows the detection and accurate knowledge of amplification product in every cycle of the reaction. Measuring kinetics at exponential phase allows the samples to be analyzed before reaction approaches the plateau phase [26].

In regular PCR, the end point varies from sample to sample and gel is insensitive to detect these variabilities, while Real – Time PCR is sensitive enough to detect them. Moreover, conventional PCR method is sophisticated, labor intensive and results are obtained within many days, also gel resolution is poor about ten folds. Whereas, Real–Time PCR is a speedy process and results can be obtained within hours and it can detect as little as two-fold change [27].

Conclusion and Future Recommendations

Our study reveals the clinical significance of genotyping of HCV patients and the accurate methods for the assessment of viral load. It is hoped that this study will help clinicians to find precise viral load in HCV positive patients and manage a better treatment plan based on correct and reliable genotyping of patients. In this we have observed that 95% of patients are infected with genotype 3a and only 5% patients were infected with genotype 1a. This data shows that genotype 3a is common in Pakistan's population which can be timely treated. Furthermore, it is clear from these results that Real–Time PCR was more accurate and reliable method for HCV genotyping and measuring the amplified product.

Acknowledgement

The authors would like to thank Dr. Bushra Uzair for her support and proofreading this paper.

References

1. Re VL, Kostman J. Management of chronic hepatitis C. Postgrad Med J. 2005;81(956):376-82.
2. Choo Q, Weiner AJ, Overby LR, Kuo G, Houghton M, Bradley DW. Hepatitis C virus: The major causative agent of viral non-A, non-B hepatitis. Br Med Bull. 1990;46(2):423-41.
3. Zein NN. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev. 2000;13(2):223-35.
4. Lavanchy D. The global burden of hepatitis C. Liver Int. 2009;29(s1):74-81.
5. Guerra J, Garenne M, Mohamed MK, Fontanet A. HCV burden of infection in Egypt: Results from a nationwide survey. J Viral Hepat. 2012;19(8):560-7.
6. Mohamoud YA, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: A systematic review and data synthesis. BMC Infect Dis. 2013;13(1):288.
7. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect. 2011;17(2):107-15.
8. Umer M, Iqbal M. Hepatitis C virus prevalence and genotype distribution Comprehensive review of recent data. World J Gastroenterol. 2016;22(4):1684.
9. Choo QL. Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-a, non-b viral hepititis genome. Science. 1989;244(4902):359.
10. Chevaliez S, Pawlotsky JM. HCV genome and life cycle. Hepatitis C viruses: Genomes and molecular biology. 2006:5-47.
11. Pybus O, Drummond AJ, Nakano T, Robertson BH, Rambaut A. The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: A Bayesian coalescent approach. Mol Biol Evol. 2003;20(3):381-7.
12. Moriishi K, Matsuura Y. Mechanisms of hepatitis C virus infection. Antivir Chem Chemother. 2003;14(6):285-97.
13. Hardy N, Sandroni S, Danielson S, Wilson WJ. Antibody to hepatitis C virus increases with time on hemodialysis. Clin Nephrol. 1992;38(1):44-8.
14. Niu MT, Coleman PJ, Alter MJ. Multicenter study of hepatitis C virus infection in chronic hemodialysis patients and hemodialysis center staff members. Am J Kidney Dis. 1993;22(4):568-73.
15. Hayashi J, Yoshimura E, Nabeshima A, Kishihara Y, Ikematsu H, Hirata M, et al. Seroepidemiology of hepatitis C virus infection in hemodialysis patients and the general population in Fukuoka and Okinawa, Japan. J Gastroenterol. 1994;29(3):276-81.
16. Poynard T, Philippe M, Ching-Lung L, Dominique G, Renée P, Marie-Hélène T, et al. A comparison of fibrosis progression in chronic liver diseases. J Hepatol. 2003;38(3):257-65.
17. Bari A, Akhtar S, Rahbar MH, Luby SP. Risk factors for hepatitis C virus infection in male adults in Rawalpindi–Islamabad, Pakistan. Trop Med Int Health. 2001;6(9):732-8.
18. Akhtar S, Moatter T. Intra-household clustering of hepatitis C virus infection in Karachi, Pakistan. Trans R Soc Trop Med Hyg. 2004;98(9):535-9.
19. Janjua N, Nizamy M. Knowledge and practices of barbers about hepatitis B and C transmission in Rawalpindi and Islamabad. J Pak Med Assoc. 2004;54(3):116-8.
20. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318-27.
21. Manns M, Wedemeyer H, Cornberg M. Treating viral hepatitis C: Efficacy, side effects, and complications. Gut. 2006;55(9):1350-19.
22. Simmonds P, Jens B, Christophe C, Gilbert D, Nobuyuki E, Stephen F, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42(4):962-73.
23. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: A systemic review. Virol J. 2011;8(1):433.
24. Kanwal S, Mahmood T. Occurrence of genetic modifications in core, 5 UTR and NS5b of HCV associated with viral response to treatment. Virol J. 2014;11:171.
25. Soheili Z, Samiei S. Real time PCR: Principles and application. Hepatitis Monthly. 2005;5(3):83-7.
26. Martell M, Gómez J, Esteban JI, Sauleda S, Quer J, Cabot B, et al. High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. J Clin Microbiol. 1999;37(2):327-32.
27. Pfaffl MW. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res. 2001;29(9):e45.