GEOGRAPHIC DISTRIBUTION OF HCV GENOTYPES IN AREAS OF PUNJAB AND KP

By

Rohina Arif

DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF BIOLOGICAL SCIENCE

QUAID-I-AZAM UNIVERSITY

ISLAMABAD, PAKISTAN

2017

GEOGRAPHIC DISTRIBUTION OF HCV GENOTYPES IN AREAS OF PUNJAB AND KP

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy in Biotechnology

By

Rohina Arif

Reg. No: 022715113025

Supervised by

Dr. Javaria Qazi

FACULTY OF BIOLOGICAL SCIENCE

QUAID-I-AZAM UNIVERSITY

ISLAMABAD, PAKISTAN

2017

CERTIFICATE

This thesis submitted by Rohina Arif is accepted in its present form by the Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as fulfilling the thesis requirement for the degree of Master of Philosophy in Biotechnology.

Supervisor:

Dr. Javaria Qazi
Assistant Professor
Department of Biotechnology
Quaid-i-Azam University, Islamabad

External Examiner:

Dr. Shamim Akhtar
Associate Professor
Department of Zoology
PAMS,Arid Agriculture University,
Rawalpaindi

Chairman:

Dr. Muhammad Naeem
Associate Professor
Department of Biotechnology
Quaid-i-Azam University, Islamabad

Date: 24th August 2017

DECLARATION OF ORIGINALITY

I hereby declare that the work accomplished in this thesis is my own research effort carried out in the Molecular Virology Laboratory, Department of Biotechnology, BioTech Laboratory Rawalpindi and is written and composed by myself and without any aid other than those mentioned herein. Any ideas taken directly or indirectly from third party sources are indicated as such. I further certified that the material includes in this thesis have not be used in part or full in a manuscript already submitted or in the process of submission in partial/ complete fulfillment of the award of any other degree from any other institution. I am solely responsible for the content of this thesis and I own the sole copyrights of it.

Signature: 

Name: Rohina

This thesis is dedicated to my parents and teacher their selfless support made it possible

ACKNOWLEDGMENTS

I would firstly like to express gratefulness to Allah Almighty who gave me an opportunity to explore my natural abilities, opportunity to study in Biotechnology and blessed me with the courage to complete this desired task in the required time frame.

I would like to express my very special appreciation and deep gratitude to my worthy supervisor Dr. Javaria Qazi, Assistant Professor, Department of Biotechnology, for her valuable guidance and unreserved devotion throughout the course of my research and during the write up of my thesis. I am profoundly grateful to her for giving me opportunity and freedom to research. She is a continuous source of inspiration, help, and motivation. To me, she is my spiritual mother. Thanks for taking care of me

I wish to express my sincere thanks to Dr. Muhammad Naeem Chairman Department of Biotechnology, Quaid-i-Azam University Islamabad for his guidance, support, and kindness throughout my degree.

I am grateful to all my teachers who enlightened my life with knowledge and u nderstanding.
I cannot say a single word of thanks to my Father and Mother. To me, there is no word discovered so for to say thanks to you.I just say I love you both!
I am grateful to Mrs. Zahida Haider and Mr. Qaiser to give me the opportunity for conducting research in their BioTech Lab Rawalpindi. I specially acknowledged the help and guidance of Mrs. Zahida
Many people contributed to making our lab pleasant workplace. I am extremely thankful to Syed Jalal Shah), Saima Mushtaq , Khulla Naseer, Noreen Malik and Tariq Jamil.
Shafaq Mustafa, Rimsha, made me forget tensions related to me and our society. We spent a good time which we always remember.
I am greatly thanking full to my friend Narmeen she always helps whenever I need her.
I express thanks to Sania Zaheer for helping me in data compilations. I am really grateful to lab technician Abdul Ghafoor for helping me throughout my research work. I wish to express thanks to Miss Ayesha Anjum for suggestions she gave me during my BS and Mphil.

LIST OF CONTENT

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

%	Percentage
µl/ µg	Microliter/Microgram
bp	Base pair
cDNA	complementary DNA
DAA	Direct acting antiviral
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetate
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
IDVU	Intravenous drug users
$MgCl_2$	Magnesium chloride
ml	Milliliter
mM	Millimolar
M-MLV enzyme	Maloney-murine Leukemia virus reverse transcriptase enzyme
NS	Non-Structural
^{o}C	Centigrade
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
rpm	Revolution per minute
RT	Reverse Transcriptase
TBE	Tris-Borate-EDTA
WHO	World Health Organization

PWID	People who inject drug
NSP	Non structural protein
HCV	Hepatitis C Virus
UTR	Untranslated region
ORF	Open reading frame

ABSTRACT

Hepatitis C virus (HCV) is a life-threatening virus that infects 177 million people globally. The virus has high prevalence and persistent HCV infection shows potentially serious complications. A severe form of infection leads to cirrhosis, hepatocellular carcinoma, and end-stage liver disease. All HCV genotypes have their particular geographical distribution patterns and a slight change in viral population has been observed in some parts of the world. The genotype of the infecting quasispecies plays an important role in the investigation of many aspects of HCV infection including epidemiology, pathogenesis, and response to antiviral treatment. This study was conducted to assess the various HCV genotype frequencies in infected patients from districts of Khyber Pakhtunkhwa and Punjab and their possible route of transmission. Serums samples of 610 randomly selected patients subjected to reverse transcriptase (RT-PCR) and nested PCR were included in this study. The age distribution ranged from 12 to 80 years with mean age of 43.6 years. The frequency of different genotypes among patients was assessed according to gender, age, and geographical region. Out of 610 serum samples, type specific fragments were observed in 544 samples. Genotypic distributions of the typable sample were determined as follows: 510 (72.41%) Genotype 3a, 34 (5.61%) Genotype 3b, 13(1.93%) Genotype 2a, 51 (8.71%) 3a+3b mix genotype, 1(0.15%) genotype 4. HCV RNA positive sample was not typed in 31 (5.57%) patients by this genotyping system. In Punjab and Khyber Pakhtunkhwa, genotype 3a was the most frequent. This genotype 3a was highly prevalent in female patients above 40 years of age. Most of the infected patients had a history of surgery and shaving by barbers. This set of data is a slight indication of genotypic distribution in few districts of Punjab and KP and endorse the results of previous studies. However, nationwide studies are required to access the actual genotypic diversity of HCV in the country.

CHAPTER 1

INTRODUCTION AND REVIEW LITERATURE

1.1.Viral hepatitis

Hepatitis is an inflammatory liver disease caused by certain drugs, toxins, some liver diseases, the heavy amount of alcohol intake; bacterial and viral infections. (Protzer *et al.*, 2012). Infection with five different viruses is the cause of viral hepatitis (Liang *et al.*, 2000). These viruses are Hepatitis E virus (HEV), Hepatitis C virus (HCV), Hepatitis A Virus (HAV), Hepatitis B virus (HBV) and Hepatitis D virus (HDV).

Viral hepatitis is considered as one of the big medical issue on the earth influencing thousands of individuals around the world. Viral hepatitis is one of the significant reasons for death and dismalness universally in the human population. The mortality rate of viral hepatitis is high due to acute infection in case of hepatitis B, C, and D which leads to the chronic active hepatitis and cirrhosis. Hepatocellular carcinoma has 5.6% of total cancer prevalence worldwide and hepatitis B-mediated HCC is more common than HCV mediated carcinoma (Owiti *et al.*, 2015). The high proportion of HCV-related hepatocellular carcinoma differs with a diverse geographical area of the world.

1.2. Hepatitis C Virus

A form of hepatitis was recognized over 30 years ago which was not hepatitis A or hepatitis B. This form of hepatitis named as non-A non-B hepatitis. Before the discovery of HCV in 1989 by Choo screening test for transfusion-associated hepatitis indicated that the cases of post-transfusion hepatitis still in significant number and caused by one other unknown infectious agent. With the help of this test, it was cleared that HCV was the cause of non-A, non-B Hepatitis (Pawltosky *et al.*, 2015). HCV was identified by antibody screening of a phage library in 1998.

It is estimated by WHO (2016), 177 million people around the world have HCV infection with the annual rate of 0.7 million. HCV is commonly characterized as 'silent pandemic' by economist intelligence unit (EIU) because it is asymptomatic and cannot be

recognized at early stages (Lemoine *et al.*, 2013). Early diagnosis of HCV can help in the avoidance of major health problems, but awareness and treatment are very low in third world countries. WHO termed HCV as 'viral time bomb due to its high prevalence, asymptomatic, low diagnosis and slowed treatment (Csete *et al.*, 2008).

HCV belongs to family Flaviridae and genus Hepacivirus. HCV is a small circular enveloped, positive-sense and single-stranded ribonucleic acid (RNA) virus with a diameter of 50nm. The total length of RNA genome is about 9000 nucleotide in length with one open reading frame (ORF) which harboring 10 viral proteins. 5 and 3 untranslated regions (UTRs) at both edges of the open reading frame (Wan *et al.*, 2016). 5 UTR helped in genotyping and evolutionary studies because it is a conserved part of HCV genome, (W J *et al.*,2015). ORF encodes a polyprotein, consisting 10 viral proteins named as Core (C), E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Fig. 1). Three structural proteins (C, E1, and E2) while P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B are 7 non-structural proteins. (Wu *et al.*, 2016).

1.3. Epidemiological characteristics of HCV

It was estimated by WHO, about 177 million people around the world infected with hepatitis C and prevalence is 2.5% (Petruzziello *et al.*, 2016). Prevalence of chronically infected children is still unknown. The prevalence of HCV infection varies clearly in the geographical area. The estimation of WHO is based on the number of people exposed to the virus and HCV antibody positive test (Gower *et al.*, 2014).

In Central Asia 5.4% the prevalence of anti-HCV, 5.3% in Central Africa, 4.2% Western Africa, 3.3% in Eastern Europe, 3% in the Midwest of North Africa region. The region where prevalence is at intermediate level includes Australia (1.4%), Southern Sub-Saharan Africa (1.3%), Central Europe (1.3%) and Latin America (1%-2%). The region which has been low prevalence is Western Europe 0.9%, Caribbean 0.8%, Oceania with 0.1%. The countries which have the highest rates of HCV are Egypt 22.1%, Pakistan 4.9%, and China 3.3%. Available data about HCV prevalence is in 138 countries while 25 countries have the

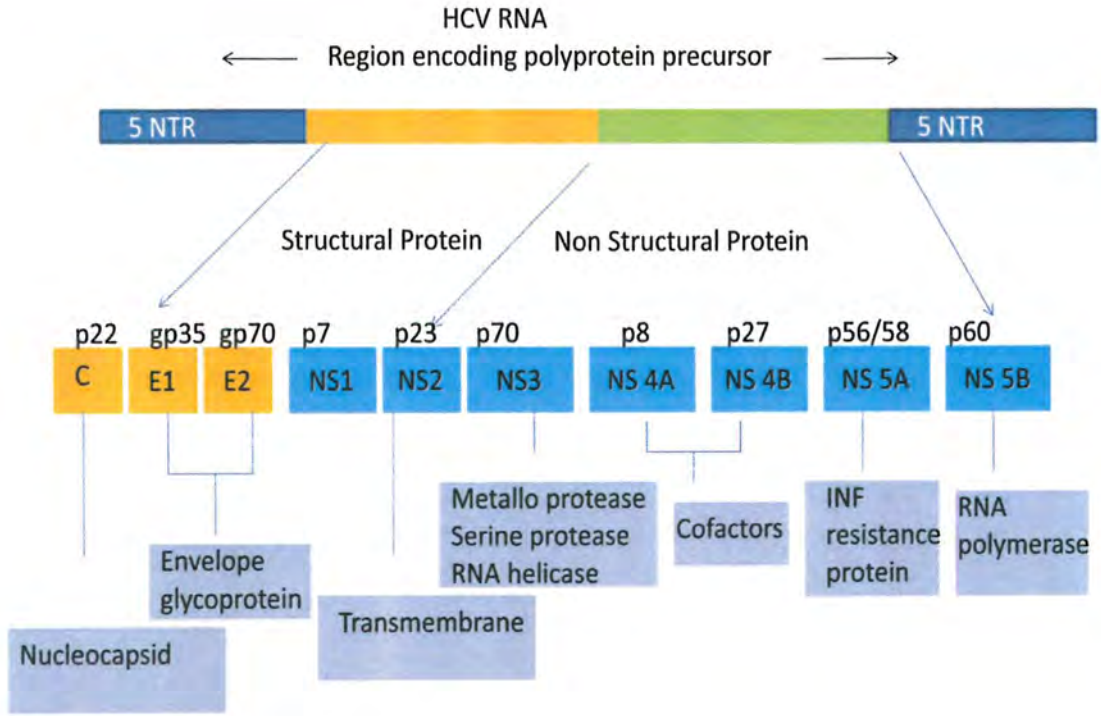


Figure: 1.1. HCV genome and protein function

This image is originally taken from Idress, 2006 and reconstructed by Coral Draw X7.

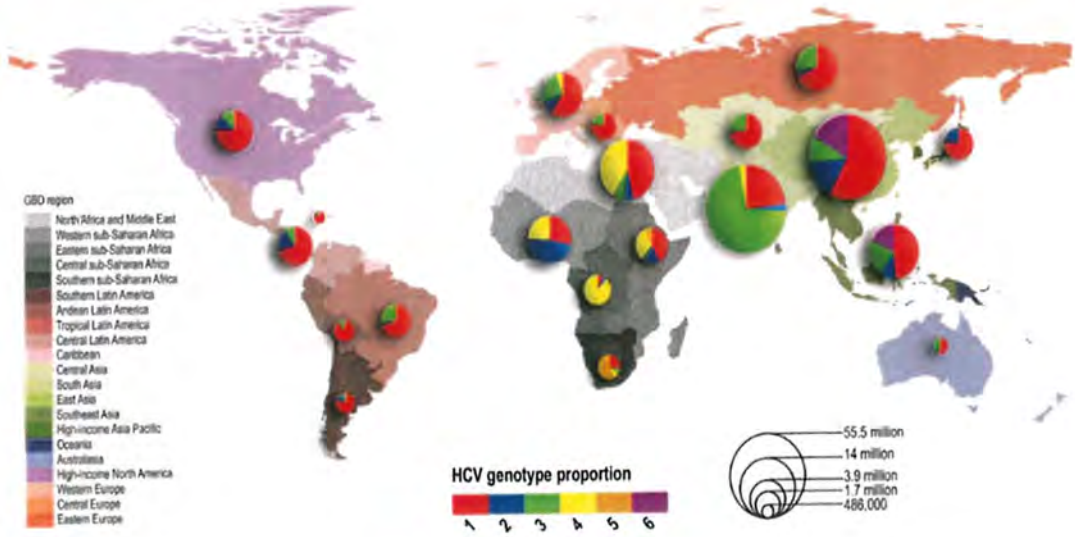


Figure: 1.2. Relative prevalence of each HCV genotype by GBD region

Size of pie charts is proportional to the number of seroprevalent cases as estimated by Hanafiah *et al.*2013.

highest proportion almost half of the total infections. Most of the world 70% infection is in China, India, Pakistan, Egypt, Nigeria, and Russia. HCV prevalence is not available in 19 countries, 4 in Asia, 4 in Americas, 5 in North Africa/Middle East Area and 6 in Europe. No data available about HCV genotypic frequency present in Malaysia, Bangladesh and North Korea (Petruzziello *et al.*, 2016).

It has been observed from 1995 to 2005 the prevalence of HCV decreased from 2.8% to 2.5% and the number of HCV-infected patients decreased from 185 to 177 million (Hanafiah *et al.*, 2013). A huge increase observed in some low-income areas such as Central Asia (+2.0%) and Central Africa (+3.7%) since the most significant decrease has been reported in the high-income zones, especially in (-1.5%) Western Europe, (-1.2%) Southern Africa and (-0.9%) Australasia (Gower *et al.*, 2014).The most prevalent genotype globally is Genotype 1 (49.1%) followed by Genotype 3 (17.9%), Genotype 4 (16.8%), Genotype 2 (11.0%), Genotype 5 (2.0%) Genotype 6 (1.4%) and Undefined or mixed genotypes (1.8%) of the total HCV infections (Petruzziello *et al.*, 2016).

Genotype 1 and Genotype 3 together causes almost 67% of total HCV infection, other genotypes are high proportion in underdeveloped countries, as Genotype 2 in West Africa (62.9%) and in few regions of South America, possibly caused by migration (Smith *et al.*, 2014).In Central and Northern Africa, genotype 4 and genotype 6 are highly present (82.9% and 65.3%).The high prevalence of genotype 4 and genotype 6 in Southeast Asia (30.8%).Infections in a few states of former USSR and Northern Europe and in Italy are caused by genotype 2 (Petruzziello *et al.*, 2016).

The high proportions of genotype 1is found in 83.0% the Caribbean, 74.3% Latin America, 66.3% North America, and Europe (64.4%) respectively. Infections in North Africa and the Middle East caused by genotype 4 (65.3%) and high proportions of genotype 4 in Egypt (93.1%). If Egypt is excluded from this region the percentage of genotype 4 is 32.3% of all infections and then genotype 4 dominated in this region 48.3%. In Asia has a large genotype 1 and genotype 3 population (46.6% and 22.4%) while in South Asia the most prevalent genotype 3 (66.7%). In Pakistan and India, the common genotype is genotype 3a (54.4% and79%) which shows the percentage in the Asia (Petruzziello *et al.*, 2016). The genotype (55%) and genotype 3 (25.5%) are high proportion in Australasia.

1.4. Epidemiology of HCV in Pakistan

HCV infection is a critical health issue in Pakistan. The world sixth densely populated country, improper health care system and poor hygienic and so the chances of acquiring the coinfection and superinfection are very high. Scientist, doctors, and patient tried to limits it Pakistan is the second highest endemic country for HCV. Around 10 million were screened and 4% seroprevalence of HCV was reported to be (Saeed *et al.*, 2014; Saleha *et al.*, 2014; Ali *et al.*, 2014).

Waheed and associates concluded from the results of ten studies that HCV is 4.95 % prevalent in general adult population. Blood donors can also be compared to the healthy population because blood donors are often the healthier among the community (Waheed *et al.*, 2009). High prevalence of HCV was reported in South East Asian countries while in Pakistan its prevalence was estimated to be 6.7 % and 1.3 % in women and children (Khan *et al.*, 2010).

HCV prevalence was reported in Punjab 6.7 %, followed by Sindh 5 %, Baluchistan 1.5 % and Khyber Pakhtunkhwa (KP) 1.1 %. In males, the high prevalence was reported as compared to female. In people with ages greater than 60 years, HCV is more common, followed by people with ages falling between 40-60 years (Basit *et al.*, 2014).
HCV prevalence is not consistent across the country; many regions have the higher prevalence. For example, in Mardan HCV positive serology has been reported as 11.7 %, Lahore 15.9 % and Gujranwala it is even higher, 23.8 % (Ali *et al.*, 2014). When compared with HCV serofrequency of neighboring countries like India (0.66%), Iran (0.87%), China (1%) and Afghanistan (1.1%), HCV serofrequency is quite high (4.7%) in Pakistan (Khan *et al.*, 2011).

Knowledge about the genotype of infecting virus is indispensable for understanding disease progression, treatment and response to anti-viral therapy and is of immense importance to curb the disease effectively. Genotype 3a is the most prevalent genotype followed by 3b and majority of population studies (Idrees and Riazdduin 2008).

The commonest HCV genotype in Pakistan is genotype 3 with 79.43 % prevalence. In their review, Khan, and co-authors concluded that among all observed genotypes the genotype 3a is the most overriding genotype with highest prevalence rate followed by genotype 1a, 3b, and mixed genotypes. Genotype 3 is ubiquitous in all regions of Pakistan with highest overall prevalence. The positive side of the dreadful picture is that genotype 3 can be to curb easily with a shorter treatment duration (Khan *et al.*, 2011). From 2010 to 2014 seventeen reports of HCV genotype distribution in Pakistan were published (Table 1.1)

1.5. Importance of epidemiology

Epidemiological studies help doctor physicians to get the root of health problem and outbreak in a population. And it also prevents from future outbreaks by vaccination. Epidemiological study helps to create awareness about hygiene and take preventive measures. The evaluation of national HCV prevalence and modes of transmission open the door for national authorities to give importance to preventive measures. The roles of risk factors and lifestyle conditions linked to HCV spread in worldwide. Epidemiological studies on the role of potential risk factors such as injections for medications, medical procedures, immunizations, intravenous drug user, tattooing, and scarification method, have shown wide geographical disparity with the major indication for local populations and potential prevention and control programs (Lavanchy 2011).

In order to lower HCV burden; prevention programs are needed. Such projects need to guarantee that donated blood is free of contamination, and that protected infusion techniques are used. In developing countries the use of non-reusable injections for immunization is crucial. Risk-education counseling for professionals and the public is of vital importance to decrease the HCV infections. Medicinal services experts and people, in general, should be made aware of transmission risk (HCV, HBV, and HIV) by contaminated injection and other medical equipment, as well as by traditional medical therapeutics or practices (Zanetti and Remo 1999) and ought to get proper. Counseling and guidance regarding the significance of controlling

Table: 1.1. Frequency of genotypes distribution in Pakistan

1		2		3		4		5	6	Mixed	Untypable	Sample	References
1a	1b	2a	2b	3a	3b	4a	4b	5a	6a				
3.3	0.8	2.1	0.2	61	8.9	--	---	0.1	0.1	4.7	17.8	20,552	Butt et al 2010
12.1	1.2	1.2	0.4	64.5	6.4	6.7	0.6	0.5	0.4	3.2	2.4	6048	Ijaz et al 2011
5.3	5.1	24.9	6.1	39.4	6.3	2.4		2.4	2	-	5.7	2941	Khan et al 2014
3.5	0.8	1	--	88.1	3	--	--	--	--	3.6	--	1537	Aziz et al 2013
7.4	5.8	13.2	6.6	26.4	16.5	-	-	-	-	4.1	17.4	1419	Ali et al 2014
23.6	-	-	-	55.9	3.2	12.5	1.2	--	--	1.2	2.5	1364	Ahmad et al 2010
2.6	0.8	0.3	0.2	82.6	0.2	---	--	0.1	-	2.4	10.8	995	Afridi et al 2014
4.3	--	--	--	73.9	13	--	---	--	--	4.3	4.3	824	Khan et al 2011
0.5	1.5	10.3	1.2	68.3	2.6	--	---	--	--	--	15.6	736	Afzal et al 2014
6.1	6	15.1	6.1	42.3	12.1	-	-	-	-	-	12.1	590	Rauf et al 2011
0.9	0.9	7.4	0.9	66.1	2.6	--	--	--	--	2.2	18.8	537	Khan et al 2014
0.72	0.72			80.26	6					6.73	27.88	415	Ali et al 2010
8.8	3	6.5	1	45.5	16	0.8	--	--	--	16	2.2	400	Muhammad et al 2011
14.3	3.6	8.9	1.8	32.1	17.8	1.8	--	---	--	10.7	8.9	384	Khan at al 2011
2.9	1.5	--	--	70.3	5.5	--	--	--	--	2.6	15.1	344	Akhund AA et al 2014
1.3	0.7	--	--	82.1	13.9	--	--	--	--	2	--	320	Nabi et al 2013
6.8	4.6	1.3	--	54.4	8.2	--	--	--	--	8.2	16.4	305	Ali et al 2011

such diseases in all therapeutic, surgical and dental clinics, safe injection practices, suitable cleansing procedures, and high-level disinfection, avoiding the re-utilizing and sharing of contaminated equipment supplies, and evading contamination of multi-utilized supplies, such as medication vials (Perz *et al.*, 2010).

1.6. Genotypic variation in HCV

HCV is characterized by a high degree of genetic diversity (Choo *et al.*, 1991) and it is similar to RNA viruses. The most heterogeneous region of HCV genome is E1 and E2. The first hypervariable region (HVR1) is the most diverse region present in the N-terminus of E2 gene and the second hypervariable region is present 3′ of HVR1(Weiner *et al.*, 1991). Genotypes, subtypes, isolates quasi-species are four hierarchical strata on which HCV has been classified. The nucleotide sequence variability of HCV has many genotypes and subtype which is related to high rate of replication and inability of RNA polymerase to proofread the sequence. HCV strains differ 30-35% nucleotide variation is classified into different genotype. HCV strains belonging to same subtype differ < 15% degree nucleotide variation (Messina *et al.*, 2014). A total of six major genotypes, named according to the order of their discovery1—6 have been identified (Smith *et al.*, 2014) and over 67 subtypes have been described (a,b,c) on the amount of nucleotide variation (Niu *et al.*, 2016).

The genotype which discovered yet are genotype 1a,1b, genotype 2a,2b,2c,2d, genotype3a,3b,3c,3d,3e,3f genotype 4a,4b,4c,4d,4e,4f,4g,4h,4i,4j, genotype 5a,genotype 6a. Different genotype has different geographical location as mention above. Some HCV subtypes are widely distributed across the globe so they are known as an epidemic subtype. Theses epidemic subtypes account for the comparatively large proportion of HCV infections. These include 1a, 1b, 2a and 3. In contrast, some are "endemic strains" confined to specific regions for a longer period (Smith *et al.*, 2014).

Quasispecies is 91-99% similar to the conserved region but different in more variable regions HVR1 and HVR2. The role of these Quasispecies populations is twofold. The first role of Quasispecies is increased viral fitness due to high mutation rates. Second, Viral Quasispecies escape from host immune system and viral persistence (Domingo *et al.*, 2012). The high

variability of the HCV virus has the biggest problem for the development of an effective vaccine or antiviral drug.

1.7. Common modes of transmission of HCV

HCV is transmitted by multiple routes.HCV is transferred by transfusion of blood, blood products, and the transplantation of organs and via the sharing of contaminated needles (Karoney *et al.*, 2013). HCV is a blood-borne infection which is transmitted Iatrogenic, sexually, vertically; occupational, cultural and recreational activities. The Iatrogenic transmission includes therapeutic injections, unsafe transfusions acupuncture. Recreational and cultural activities include Intravenous drug use, scarifications, tattooing, ear piercing may spread HCV it may also be transmitted by needle-stick injuries (Lavanchy, 2011).

1.8. Objectives

- Patient sample collection and compilation of demographic profile.
- Optimize genotyping of HCV using patient sera.
- Analysis of genotypic variation from random samples received from cities of Punjab and KP.
- To determine major risk factors based on patient's demographic profile.

CHAPTER 2

MATERIALS AND METHODS

2.1. Sample and acquisitions of data

This research work has been done in collaboration with BioTech Lab Rawalpindi. The total of 610 HCV-positive serum samples were received from the two provinces of Pakistan through our collaborator in Islamabad, Rawalpindi Sargodha, Faisalabad, Peshawar, Mardan, Swabi, Gujrat and Jhelum. The period of research is 4th August 2016 to 1st June 2017. The age of patients was between 12 to 80 years. All the samples were stored at -70°C.

2.1.1. Demographic profile

The total number of samples were 610 out of which 568 (93.11%) were from Punjab, 42 (6.8%) samples from Khyber Pakhtunkhwa, There were 265 (43.44%) males and 345 (56.55%) females. The age of patient was 12 to 80 years; therefore, we distributed the samples in three groups, first age group (<20) contains 14 (2.2%) samples and second age group (21- 40) contains 261 (42. 78%).The third age group (41-60) contains 279 (45.73%).The fourth age group (60<) contain 56 (9.1%).

2.2. Viral RNA extraction

HCV RNA was extracted from 100µl serum by QIA Viral RNA Mini Kit (GenePro) with minor modifications. In ACD Vacutainers fresh blood 5cc was gathered and centrifuged at 3000rpm for 10min. In 1.5ml Eppendorf tube ,100µl serum was separated and kept at -20°C.AVL Buffer 100µl from GenePro Viral RNA Mini Kit, 20µl of Proteinase K and 7.5µl of carrier RNA was pipette into a 1.5ml Eppendorf tube. In AVL-carrier RNA buffer containing the Eppendorf tube, 100µl serum was added and mixed by pulse vortexing for 10s Incubated at room 70°C for 10mins. To get rid of drops within the cap the tube was centrifuged for 10seconds. 140µl of ethanol (96-100%) was included in it and blended by pulse vortexing for 15s. After that, the Eppendorf tube was centrifuged at 8000rpm for 10s. The prepared lysate was moved into the QIAamp Mini column and embedded in the collection tube. The column was centrifuged at 8000rpm for 1min. The step was repeated whole lysate was passed through the column. Buffer AW1 of 250µl was added to sample containing column tube and

centrifuged at 8000rpm for 1min. In Q1Amp Mini Column Buffer AW2 of 300µl was included. The cap was closed then centrifuged at 8oopm for 1min. The purification column containing sample was placed into a new collection tube and old collection tube was discarded. The column was centrifuged at 12000rpm for 2mins and left for 1min to dry. The QIAmp Mini spin column consisted of the sample was placed into a clean 1.5ml microcentrifuge tube.
the QIAmp Mini column was carefully opened and add 50µl of AVE buffer was added equilibrating to room temperature. The cap was closed and kept for 1min incubation at room temperature. Centrifuged at 8000rpm for 1min and kept at -20°C. Same protocol was used for all the HCV samples.

2.3. Complementary DNA (cDNA) synthesis

Viral RNA was reversed transcribed by utilizing M-MLV Reverse Transcriptase (Enzynomics, Catalogue # RT0015) with the slight adjustment. The total reaction volume was 20µl as given in Table 2.2. After making reaction mixture, the contents of tube was blended delicately and incubated for 10min at 25°C following by 55min at 37°C and 15min at 70°C. Finally, after incubation, cDNA was stored at -20°C. The amplified cDNA have been used as a template for amplification in PCR. The total reaction volume was 20µl as given in Table 2.2

2.4. PCR

The cDNA was amplified by polymerase chain reaction. HCV fragments were amplified in a reaction 20µl volume 2µl cDNA included. In first round, PCR 5'UTR plus core region is amplified. Outer sense primer and antisense primer were used in the 1st round PCR reaction and then inner primers were used in a 2nd round nested PCR. PCR product of 1st round was used as a template for the 2nd round nested PCR. The type-specific primers were separated into groups because we tried to detect 12 different HCV types. Therefore, in the second-round PCR the sample was separated into two mixes (Mix 1, Mix2). Mix-1 consist of primers for genotypes 2a, 3b, 2b, 3a, 2 and Mix-2 contained the 1a, 4, 3a, 5a, and 6a primers. The final reaction volume was 20µl as mentioned in Table 2.3.

2.5. Detection of PCR amplicons by agarose gel electrophoresis

1% agarose gel was prepared by weighing 0.5g of agarose powder on weighing balance and adding it in 30ml 1X TAE buffer. The crude mixture was heated in the oven for almost 2minutes, in a glass bottle dedicated to gel preparation, to get a clear gel solution. It was kept at room temperature until its temperature was dropped to 60°C.10µl Ethidium bromide was added and gel solution was poured off onto the gel casting tray (which was already affixed with the appropriate comb) and allowed to set. Gel tray containing solidified gel was placed in an electrophoresis tank containing the suitable amount of 1X TAE buffer to let the gel completely dip into it (a thin layer of the buffer should be formed on the gel surface, most precisely). 10X loading buffer (25% Ficol, 0.4% bromophenol blue and 0.4% xylene cyanol FF) was mixed with DNA samples and loaded into wells of agarose gel along with 1000 base pair (bp) Ladder (Thermo Fisher Scientific). It was run at 100V for 30-45minutes.UV transilluminator is used to visualize the PCR products on the gel. DNA fragments length was defined by comparison with the standard molecular weight that had been loaded and run on the same agarose gel.

2.6. Statistical analysis

Epi Info 7 (Center for Disease Control, GA, USA) was used for the data analysis and the summary statistic. The 95% confidence intervals (95% CI) of prevalence were expected using the Mid-p algorithm. The p values less than 0.05 were considered to be statistically significant.

Table: 2.1. Demographic profile of patient's data

Region	Female	Male	Total
Attock	2	2	4
Faisalabad	65	59	123
Gujranwala	61	41	102
Hangu	2	1	3
Islamabad	45	46	91
Jehlum	56	21	77
Jhang	4	3	7
Mardan	5	7	12
Multan	2	0	2
Peshawar	5	7	12
Rawalpindi	71	61	132
Sargodha	15	12	27
Sawabi	10	5	15
Total	345	265	610

Table: 2.2. First strand cDNA synthesis optimized concentrations

Reagents	Quantity
10mMdNTPs	2µl
dH_2O	2µl
10X Buffer RT	4µl
Primer C	1µl
M -MLV	1µl
RNA Sample	10µl
Total Volume	20µl

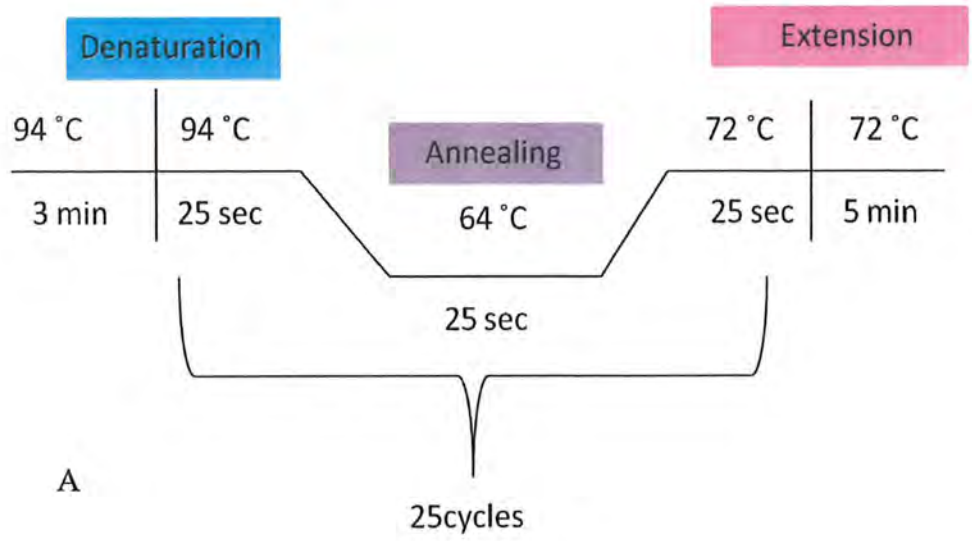
Table: 2.3. Nested PCR (Round 1)

Reagents	Quantity
25mMMgCl$_2$	2.4µl
dH$_2$O	8.2µl
10XBuffer	2µl
100mMdNTPs	1µl
Primer Forward (01)	1µl
Primer Reversed (02)	1µl
500UTaqPolymerse	0.4µl
cDNA sample	4µl
Total volume	20µl

Table: 2.4. Nested PCR (Round 2)

Mix 1		Mix 2	
H$_2$O	3.2µl	H$_2$O	3.2µl
25mM MgCl$_2$	2.4µl	MgCl$_2$	2.4µl
10XBuffer	2µl	10XBuffer	2µl
100mMdNTPs	1µl	100mMdNTPs	1µl
Primer	6µl	Primer	6µl
Taq Polymerase	0.4µl	Taq Polymerase	0.4µl
cDNA Sample	5µl	cDNA Sample	5µl
Total volume	20µl	Total	20µl

The six primers are added in each mix, each primer volume is 1µl



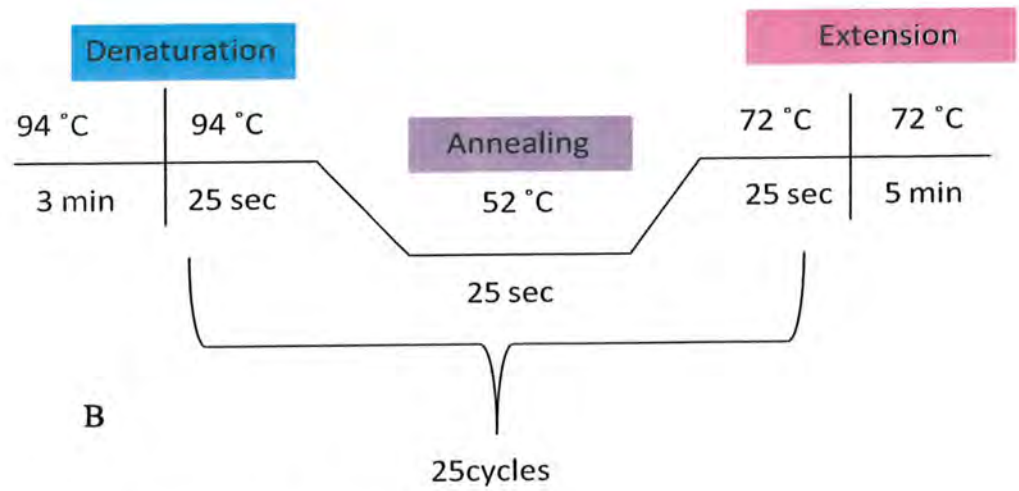


Figure: 2.1. Nested PCR conditions

A) Nested PCR conditions for round 1 ,B) Nested PCR conditions for round 2 .Figure was constructed by Coral Draw X7 showing different cycles of PCR

﴿کالے یرقان کے بارے میں سوالات وجوابات﴾

1۔خون کے نمونے کا نمبر۔۔۔۔۔۔۔۔۔۔

2۔جنس۔ مرد ☐ عورت ☐

3۔عمر۔۔۔۔۔۔۔۔۔۔

4۔ازدواجی حیثیت۔ شادی شدہ ☐ غیر شادی شدہ ☐

5۔مستقل رہائش۔۔۔۔۔۔۔۔۔۔

6۔عارضی رہائش۔۔۔۔۔۔۔۔۔۔

7۔قومیت

(1) پنجابی۔(2) سندھی۔(3) بلوچی۔(4) پختون (5) سرائیکی یا کوئی اور۔۔۔۔۔۔۔۔۔۔

شہری علاقہ ☐ دیہی علاقہ ☐

8۔خاندان کتنے افراد پر مشتمل ہے؟۔۔۔۔۔۔۔۔۔۔

9۔ایک کمرے میں کتنے افراد رہتے ہیں؟۔۔۔۔۔۔۔۔۔۔

10۔تعلیمی حیثیت۔ ان پڑھ ☐ تعلیم یافتہ ☐ فنی تعلیم ☐

11۔مذہب۔ اسلام ☐ ہندو ☐ سکھ ☐ عیسائی ☐ کوئی اور ۔۔۔۔۔۔۔

نوٹ: ہم یہ سوالات اس لئے پوچھ رہے ہیں کیوں کہ مختلف بیماریاں مختلف انداز میں ردعمل دیتی ہیں۔

12۔کیا آپ کو کوئی اور بیماری ہے؟

یرقان ☐ تھیلیسیمیا ایڈز ☐ گردوں کا مرض ☐ ہیموفیلیا ☐

13۔کیا آپ کا کبھی آپریشن ہوا ہے؟ ہاں ☐ نہیں ☐

14۔آپ کی بیماری کی تشخیص کب ہوئی؟ ۔۔۔۔۔۔۔۔۔۔

15۔کیا آپ نے مستند معالج سے مشورہ کیا ہے؟ ہاں ☐ نہیں ☐

16۔کیا آپ نے کبھی خون لگوایا ہے؟ ہاں ☐ نہیں ☐

17۔کیا کبھی کوئی چوٹ لگی ہے جس میں ٹانکے لگے ہوں؟ ہاں ☐ نہیں ☐

18۔کبھی دندان ساز (Dentist) کے پاس گئے ہیں؟ ہاں ☐ نہیں ☐

19۔بیماری کے دوران ڈاکٹر کے پاس جاتے ہیں؟ ہاں ☐ نہیں ☐

20۔گھر میں کسی کو کالا یرقان ہے؟ کس کو۔۔۔۔۔۔۔۔۔۔ ہاں ☐ نہیں ☐

21۔آپ کو کیا لگتا ہے کہ آپ کو یہ بیماری کیسے ہوئی؟۔۔۔۔۔۔۔۔۔۔

☆صرف مرد حضرات کے لئے۔

1۔کیا آپ نے نشہ کے لئے سرنج کا استعمال کیا ہے؟ ہاں ☐ نہیں ☐

2۔آپ حجام کے پاس جاتے ہیں تو اپنی کنگھی استعمال کرتے ہیں؟ ہاں ☐ نہیں ☐

3۔حجام ہر دفعہ بلیڈ تبدیل کرتا ہے؟ ہاں ☐ نہیں ☐

☆خواتین کے لئے۔

1۔کیا آپ نے کبھی ناک یا کان چھدوایا ہے؟ ہاں ☐ نہیں ☐

2۔کیا آپ بیوٹی پارلر جاتی ہیں؟ ہاں ☐ نہیں ☐

3۔بچہ گھر میں پیدا ہوا ہے؟ ہاں ☐ نہیں ☐

4۔دوران زچگی خون لگوایا تھا؟ ہاں ☐ نہیں ☐

Figure: 2.2. Patient information Performa in Urdu

PATIENT QUESTIONNAIRES: HEPATITIS C

1. Blood Sample Number: ----------
2. Sex: Male ☐ Female ☐
3. Age: ----------
4. Marital Status: Married ☐☐ Unmarried ☐☐
5. Permanent Residence: ----------
6. Current Residence: ----------
7. Ethnicity: Punjabi ☐☐ Sindhi ☐☐ Balochi ☐☐ Pakhtoon ☐☐ Siraiki ☐☐
   Others ☐☐
   Rural Area ☐☐ Urban Area ☐☐
8. How many family members do you have? ----------
9. How many individuals live in a room? ----------
10. Education: Uneducated ☐ ☐Educated ☐☐ Skills ☐☐
11. Religion: Islam ☐☐ Hindu ☐☐ Sikh ☐☐ Christian ☐☐ Others ☐☐
12. Do you have some other disease?
    Jaundice ☐ ☐☐ Thalassemia ☐☐ AIDS ☐☐ Kidney Disorder ☐☐
    Hemophilia ☐☐
13. Did you ever have medical surgery? Yes ☐☐ No ☐☐
14. When did your disease being diagnosed? ----------
15. Did you visit your specialized doctor? Yes ☐☐ No ☐☐
16. Have you ever received blood? Yes ☐☐ No ☐☐
17. Have you ever had wound followed by stitches? Yes ☐☐ No ☐☐
18. Have you ever visit dentist? Yes ☐☐ No ☐☐
19. Do you visit doctor during illness? Yes ☐☐ No ☐☐
20. Have anyone have hepatitis in family? Yes ☐☐ No ☐☐
    If yes, name the relation with the family member. ----------
21. What do you think about the cause of your disease? ----------

FOR MEN

1. Have you ever use syringe for intoxication? Yes ☐☐ No ☐☐
2. Do you use your own comb while visiting barber? Yes ☐☐ No ☐☐
3. Does barber change blade every time? Yes ☐☐ No ☐☐

FOR WOMEN

1. Have you ever pierce nose or ear? Yes ☐☐ No ☐☐
2. Do you go to beauty parlour? Yes ☐☐ No ☐☐
3. Did you give birth at home? Yes ☐☐ No ☐☐
4. Have you received blood during delivery? Yes ☐☐ No ☐☐

Figure: 2.3.Patient information Performa in English

Table: 2.5. List of HCV primer for cDNA synthesis and genotyping

C1	5′ACTGGCAAGCACCCTATCAGGCAGTAC3
G1	5′GGGAGGTCTCGTAGACCGTGCACCATG 3′
G2	5′GAGMGGKATRTACCCCATGAGRTCGGC 3′
G3	5′AGACCGTGCACCATGAGCAC3′
G4	5′TACGCCGGGGTCAKTRGGGCCCCA3′
G5	5′AACACTAACCGTCGCCCACAA3′
G6	5CCTGCCCTCGGGTTGGCTAR3′
G7	5′CACGTGGCTGGGATCGCTCC3′
G8	5′GGCCCCAATTAGGACGAGAC3′
G9	5′CGCTCGGAAGTCTTACGTAC3′
G10	5′GGATAGGCTGACCTCTACCT3′
G11	5′GCCCAGGACCGGCCTTCGCT3′
G12	5′CCCGGGAACTTAACGTCCAT3′
G13	5′GAACCTCGGGGGGAGAGCAA3′
G14	5′GGTCATTGGGGCCCCAATGT3′

CHAPTER 3

RESULTS

A total of 610 samples were collected from Punjab and Khyber Pakhtunkhwa (KP) that were screened for HCV infection by PCR. Among them, 575 samples were detected positive for HCV infection while 35 were HCV negative and 31 samples did not specific genotypic band. Of these 544 HCV RNA positive individuals, 241 (44.30%) were males and 303 (55.33%) were females. Age group 41 to 60 years was found to be more infected with HCV. Ohno *et al.* method was used for genotyping based on HCV core region-specific primers. Genotypes 2a, 3b, 3a, and were identified, and in some cases, a mixed infection was found. In our study, 4 genotypes of HCV were identified. HCV genotype 3a was in high proportion (81.80%), superseded by genotypes 3a+3b (9.375%), 3b (6.25%), 2a (2.38%) and 4 (0. 18%).Overall, the rate of HCV infection was comparatively higher in Females (55.33%) than males (44.30%) (Figure 2). Most of the patients were unaware of infection source and major causes of infection concluded from the current study were the Surgery and Barbershop.

3.1. Overall prevalence of HCV infection

In the current study, out of 610 (245 males and 365 females) patients, ranging in age from 12-80 years and above, 544 individuals (241 males and 303 females) were positive for HCV infections. Nested PCR was used for HCV RNA genotyping. .

In our subject population, 51 cases of co infection were found. Our results clearly, speculate that HCV 3a genotype was more prevalent in Punjab and KP genotype 3a was found to be most dominant HCV RNA among patient with 81.80% (n=445) prevalence abide by genotype 3a+3b with 9.375 % (n=51) prevalence, genotype 2a with 2.38% (n=13) prevalence and genotype 3b with 6.25% (n=34) genotype 4 0.1% (n=1) prevalence. Tabular representations of the results are shown in Table.3.1.

3.2. Regional prevalence of HCV infection

In our study, 544 samples were HCV positive out of which 507 (93.19%) were from Punjab and 37 (6.8%) samples from Khyber Pakhtunkhwa. In this study cities Sargodha,

Jhelum, Gujranwala, Faisalabad, Multan, Attock, Jhang, Rawalpindi and Islamabad are included from Punjab Province and from KP Peshawar, Hangu, Sawabi, and Mardan. The results are shown in Table 3.2

3.2.1. Prevalence of HCV genotypes in Punjab

To investigate the burden of HCV genotype in Punjab Five hundred and seven patients (93.19%) samples were genotyped, 282 male and 225 were female. The HCV genotype distribution from Punjab province is shown in Table 3.1. The most common genotype was 3a 421(83.03%), followed by 3a+3b mix 45 (8.8%), 3b 27(5.3%), 2a 11 (2.1%), 4 1(0.18%).

3.2.2. Prevalence of HCV genotypes in KP

The HCV distribution genotyped investigated in 37 (6.8%) subjects from Khyber Pakhtunkhwa, 20 were female and 17 males. The frequency of 3a genotype is 22 (59.45%), 2a genotype 2 (5.4%), 3a+3b mix 6 (16.21%) and 3b genotype 6 (16.21%).

3.3. Prevalence of HCV on the basis of age disparity

To evaluate the burden of disease total 610 samples have been screened for HCV by PCR. Only14 (2.4%) samples were positive for HCV infection from first age group (<20). From the second age (21 to 40 years) 248 (43.13%) samples were positive for HCV infection. In the third group age (41 to 60 years) 266 (46.26%) were positive for HCV infection. The Fourth Age group (60<) contain 53 (9.2%) positive samples. The age distribution ranged from 12 to 80 years with mean age of 43.6 years.

3.4. Gender wise distribution of HCV genotypes

To evaluate the burden of HCV disease, the total of 610 samples were collected from Punjab and Khyber Pakhtunkhwa (KP) that were screened for HCV infection by PCR. The total 544 samples were HCV positive, 241 (44.30%) and 303 (55.33%) female.

3.5. Association of HCV prevalence with marital status

When distributed on individual's marital status, out of 544 HCV-positive 369 (67.8%) were married and 175 (32.1%) were unmarried. Thus, HCV rate of infection was higher in married than unmarried.

3.6. Possible modes of HCV transmission

Detailed analysis of questionnaires filled during sampling was done to chart out potential risks related to HCV transmission. Our data depicts that 40.8% of all HCV positive patient reported that they had undergone dental or general surgery in the near past, 2.08% dialysis, 1.2% reported exposure to multiple injections, 20.1% indicated routine barber shop shaving, 2.6% indicated blood transfusion and 6% indicated the previous contact with hepatitis patient. The aforementioned results are represented in Table 3.2. All these risks are avoidable through awareness.

Table: 3.1. Region-wise distribution of genotypes

Region	HCV 2a Genotype	HCV 3a Genotype	HCV 3a,3b Mix Genotype	HCV 3b Genotype	HCV 4 Genotype	HCV untypable Genotype	Undetectable Genotype	Total
Attock	0	3	0	1	0	0	0	4
Faisalabad	2	94	11	5	1	5	6	124
Gujranwala	1	82	7	3	0	4	6	103
Hangu	0	0	1	1	0	1	0	3
Islamabad	2	57	10	7	0	11	4	91
Jehlum	2	59	3	5	0	5	3	77
Jhang	0	5	2	0	0	0	0	7
Mardan	0	7	2	2	0	0	1	12
Multan	0	2	0	0	0	0	0	2
Peshawar	0	7	2	0	0	1	2	12
Rawalpindi	3	100	10	6	0	4	10	133
Sargodha	1	21	2	1	0	0	2	27
Sawabi	2	8	1	3	0	0	1	15
TOTAL	13	445	51	34	1	31	35	610

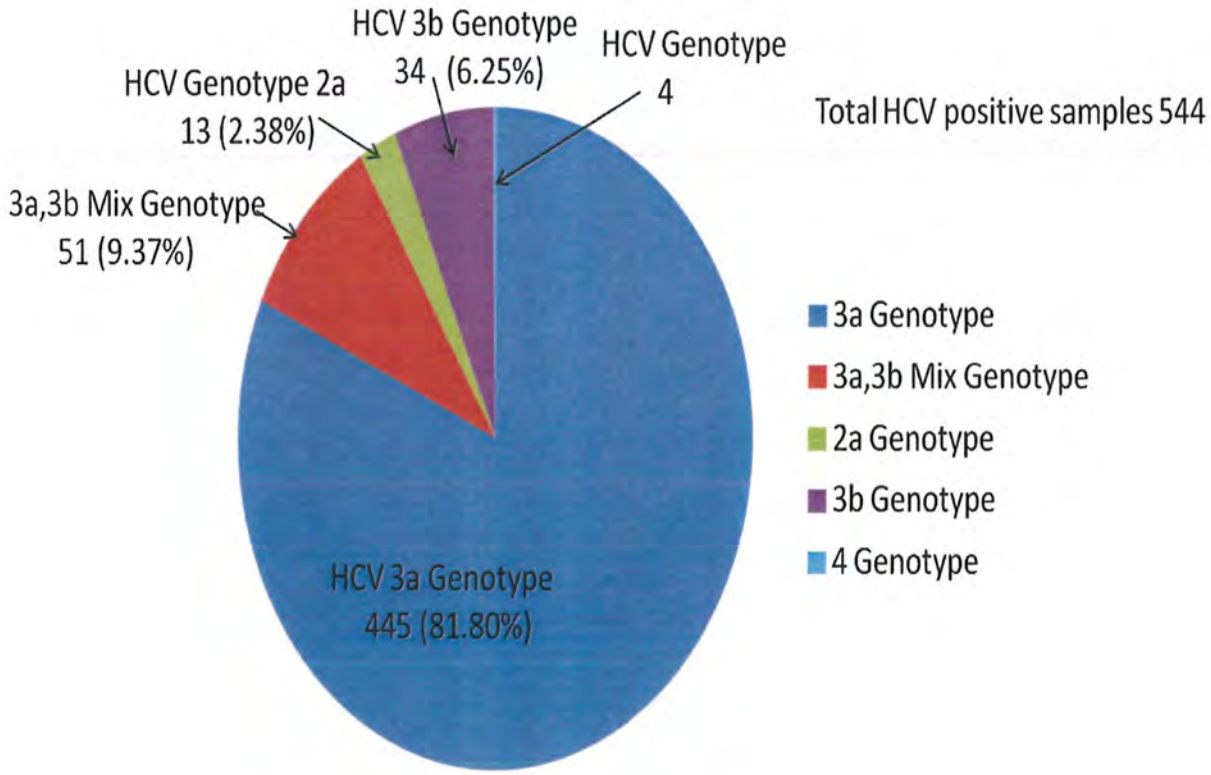


Figure: 3.1. Genotypic distribution of HCV in genotyped samples

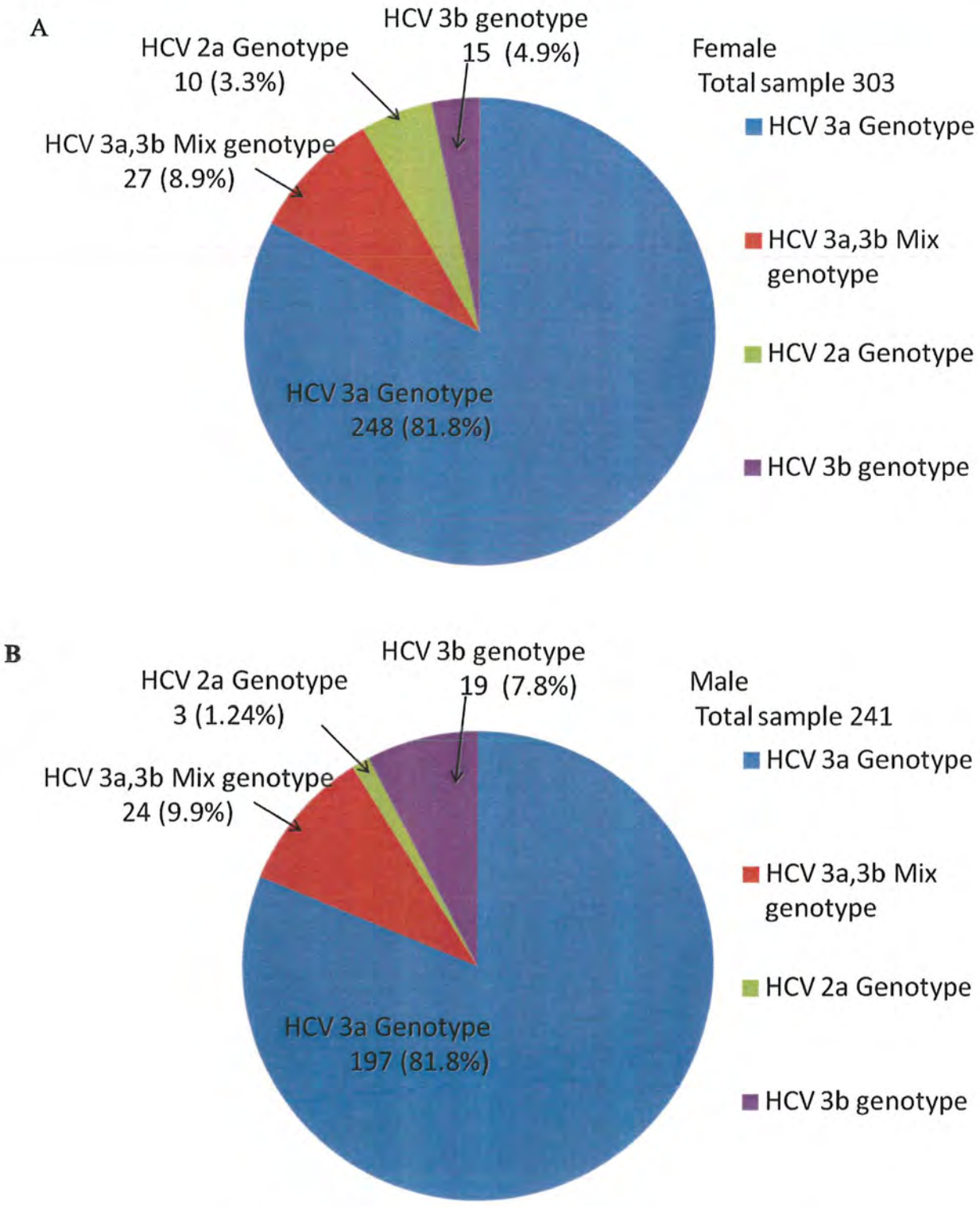


Figure: 3.2. Gender wise genotypic distribution of HCV in genotyped samples

A Female B) Male

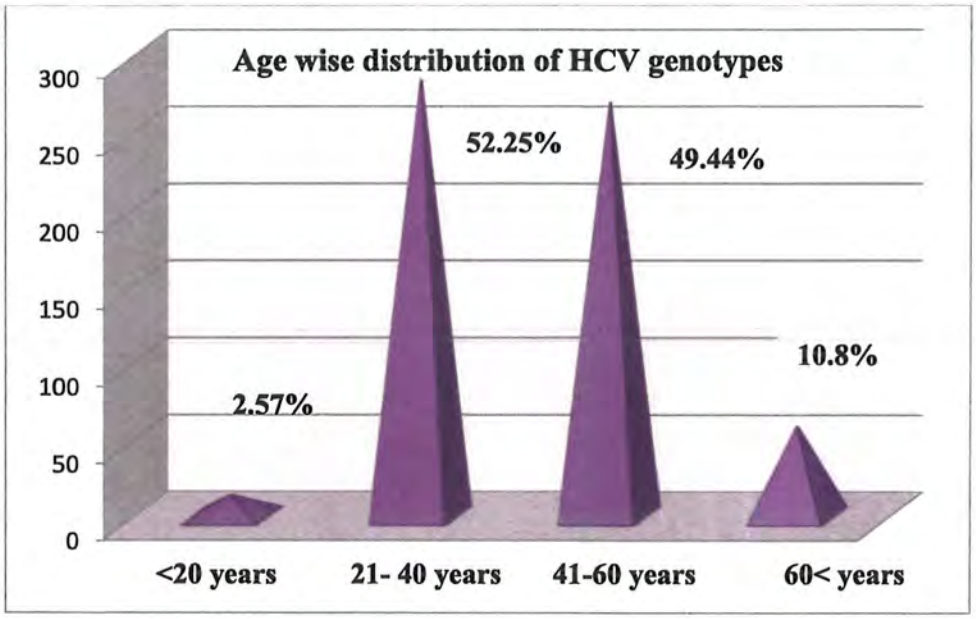


Figure: 3.3. Age wise distribution of HCV genotypes in areas of Punjab and KP

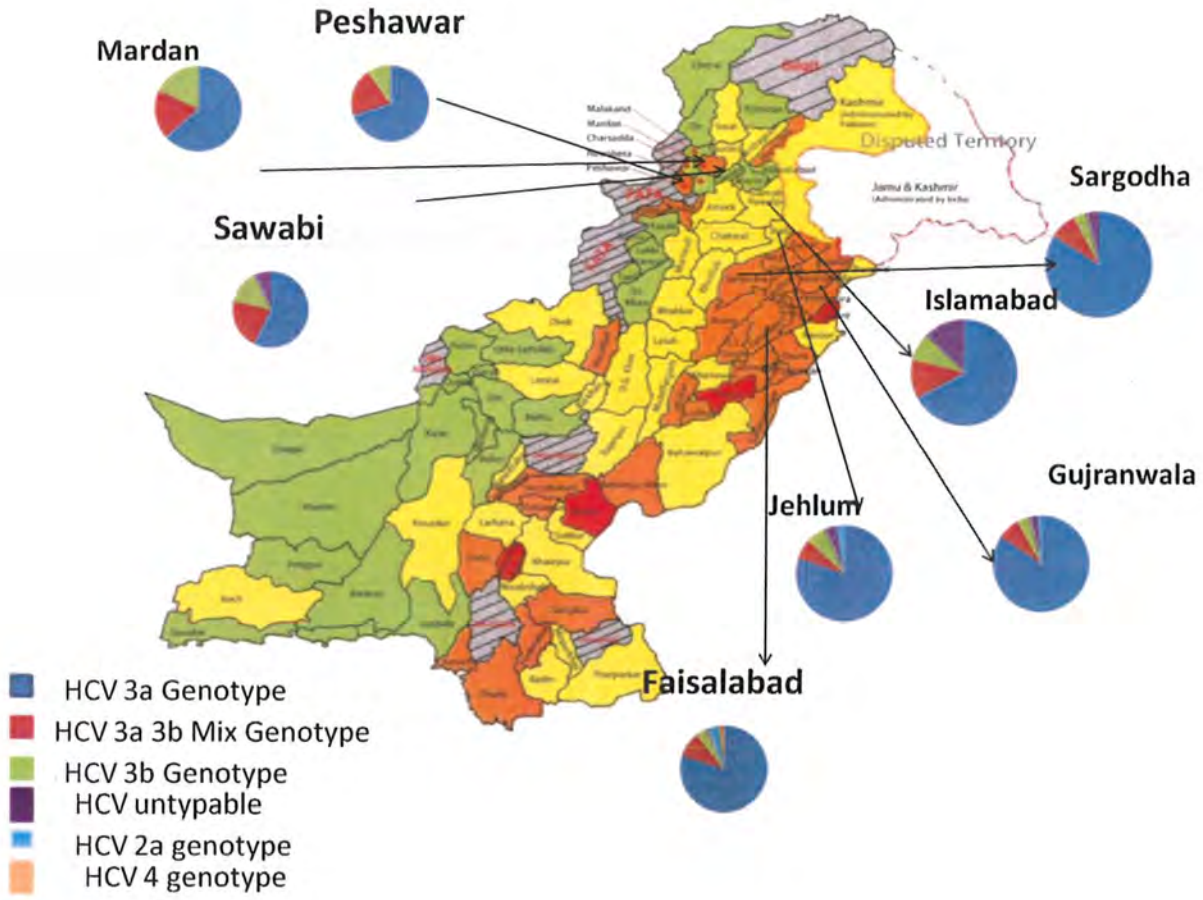


Figure: 3.4. Regional distribution of HCV genotypes

The map was constructed using Coral Draw X7.Colors showing the distribution of genotypes in Punjab and KP

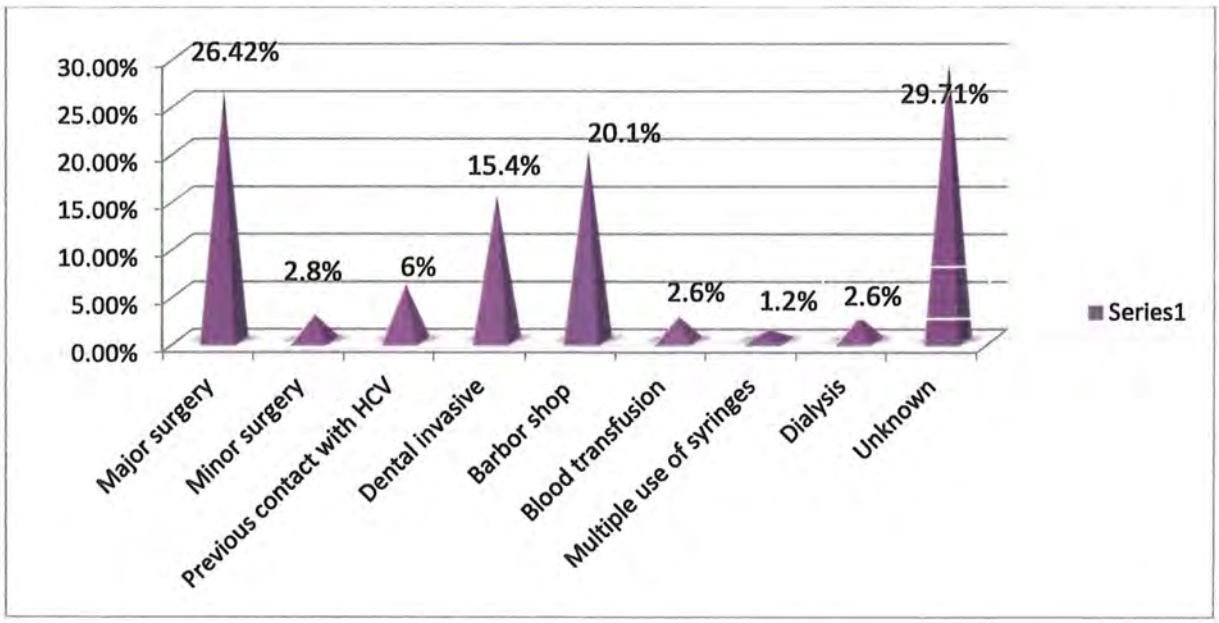


Figure: 3.5. Possible modes of transmissions

Table: 3.2. Statistics of possible modes of transmissions

Mode of transmission	Frequency
Major surgery	129
Minor surgery	17
Previous with HCV	35
Dental invasive	89
Barbor Shop	116
Blood transfusion	15
Multiples use of syringes	7
Dialysis	15
Unknown	154

CHAPTER 4

DISCUSSION

Pakistan is a country that has low socio- economic status and is highly endemic for hepatitis due to poor hygienic and improper health care system so the chances of acquiring co-infection and superinfection are quite high. The reasonable data published from Pakistan report the epidemiology of HBV and HCV from different population groups across the country (Owiti *et al.*, 2015). But in most of the reports, ELISA is used for screening of the samples. So the actual prevalence of active infection circulating community cannot be determined based on these reports. These reports also focus on specific small groups of different clinics or hospitals and do not cover the samples from the co-infection and superinfection of these blood-borne viruses in a specific cohort.

The government and concerned authorities should pay attention to the risk factor that is responsible for the spread of infection. There is also a need for conducting a vast no of epidemiological or phylogenetical studies to identify the actual prevalence of active infections and to evaluate the risk factors as well as the origin of the spread of infection. Detection of the infecting virus genotype is significant for the investigation of numerous aspects of HCV infection, including pathogenesis, response to antiviral therapy and epidemiology. The clinical status of HCV infection all over the world is elucidated by focused on the mass screening of population. The studies on the distribution of HCV genotype help in preventive strategies and treatment options (Khan *et al.*, 2014). Furthermore, distribution patterns of HCV genotypes differ considerably among different nations and areas of the same country (Messina *et al.*, 2015). Therefore, conducting studies on the distribution pattern of HCV genotypes in Pakistan is critical for better understanding of HCV infection.

In our study, we processed 610 samples with HCV infection from two provinces of Pakistan (Punjab Pakhtunkhwa) to find distribution patterns of HCV genotypes within this population. Genotype 3a, 3b, 2a, 3a+3b, 4 and untypable were identified. The most predominant genotype of HCV 3a followed by 3a+3b mix and 3b. Genotype 3a shows the wide geographic distribution in Pakistan. In India, Nepal, Bangladesh, Afghanistan genotype 3a is prevalent (Messina *et al.*, 2015). In China and Iran genotype 1a

and 1b were predominant (Nui *et al.*, 2016). These results are also in agreement with the results of the study conducted by Saleha and colleagues in their studied population of district Bannu (Saleha *et al.*, 2014). Similar results were reported by Ali and colleagues in which genotype 3a was reported to be most prevalent among all observed genotypes abide by genotype 3b (Ali *et al.*, 2014).
Inamullah and work fellows also reported the highest prevalence of genotype 3a among all observed genotypes in their studied population (Inamullah *et al.*, 2011).

The study conducted by Sajid and Ayaz in district Mardan showed that genotype 3a (26.44%) and genotype 3b (16.52%) is widespread (Ali *et al.*, 2014). In other study conducted by Idrees and Riazuddin on 3351patients, the most prevalent 3a genotype 1664 (49.7%) followed by 3b genotype 592 (17.7%), 1a genotype 280 (8.4%), 2a genotype 252 (7.5%), 1b genotype 101 (3.0%), and 4 genotype 50 (1.5%).

In our study report, 1.8% of genotype 2a was found in serum samples of infected individuals. Our results are consistent with Aziz *et al.*, who reported 1% proportion of 2a genotype (Aziz *et al.*, 2013). The 2a genotype is rare in Pakistan, but studies from bordering country India reported the low incidence of 2a genotype. This verdict is not unexpected. These less prevalent genotypes spread in Pakistan by other regions where these genotypes are endemic. In our study 1 sample from Faisalabad is genotype 4 while in a study carried out by Hafsa reports genotype 4 is absent in Pakistan.

In our study, mixed infections were observed in 8.28% of samples. In a study by Hafsa mixed genotype distribution occurred 55 (3.6%) infected persons. These details recommend that an individual infected with one genotype of HCV also has a chance to acquire other genotypes of HCV (Aziz *et al.*, 2013). We compare our findings with Idress and Riazuddin results, who report showed the proportion of mixed HCV genotype 4.8% infections in Pakistan.

In both provinces, Punjab and Khyber Pakhtunkhwa genotypes distribution of HCV was same, where the 3a genotype was predominant. In our study, distribution of HCV genotypes was same in both female and male while in Libya, gender wise variation was

found. Genotype 4 was commonly found in females, whereas genotype 1 was predominant in males (Elasifer *et al.*, 2010).

We observed that 5% of samples are untypable, which is inconsistent with previous findings that showed 20.16% samples are untypable in KP (Ali *et al.*, 2014). We observed 5% of HCV samples are untypable. In addition to that a study reported by (Butt *et al.*, 2010) based on the result of samples population size 20552 patients in a period of 2000-2009 showed that 17% samples were untypable. With the passage of time, the untypable genotype is going to increase. Quasi-species are constantly reported in infected patients. Genotyping is done from the E1 and NSB5 highly conserved part of HCV genome comparing their genetic diversity is accepted method for genotyping (Wu *et al.*, 2016). If the highly-conserved region of HCV genome mutated result in loss of genotyping capability of this method. Many factors involved in the changing pattern in HCV-like host immunological pressure, high genome mutation rate, drug force, changes in transmission route, viral/host immune escape mechanism, migration and a lot of other factors (Afzal *et al.*, 2014).

More research has been conducted to consider potential risk factors for the spread of HCV for successful execution of preventive strategies to decrease adolescent exposure to HCV infection. We concluded that the present data indicates HCV genotype 3a is the frequently present in Pakistan followed by 3b. The need for periodical investigations of HCV genotypes, knowing the distribution patterns of genotypes would provide the key information for HCV spreads as well as treatment options.

CHAPTER 5

REFERENCES

Afridi, S. Q., Zahid, M. N., Shabbir, M. Z., Hussain, Z., Mukhtar, N., Tipu, M. Y., ... & Yaqub, T. (2013). Prevalence of HCV genotypes in district Mardan. *Virology Journal*, *10*(1), 90.

Afzal, M. S., Khan, M. Y., Ammar, M., & Anjum, S. (2014). Diagnostically untypable hepatitis C virus variants: it is time to resolve the problem. *World Journal of Gastroenterology: WJG*, *20*(46), 17690.

Ahmad, W., Ijaz, B., Javed, F. T., Jahan, S., Shahid, I., Khan, F. M., & Hassan, S. (2010). HCV genotype distribution and possible transmission risks in Lahore, Pakistan. *World J Gastroenterol*, *16*(34), 4321-4328.

Akhund, A. A., Akhtar, R., Husain Naqvi, S. Q., & Kamal, M. (2014). Chronic hepatitis; HCV genotypes in correlation with alanine aminotransferase level in patients in interior of sindh. *Professional Medical Journal*, *21*(3).

Ali, A., Ahmed, H., & Idrees, M. (2010). Molecular epidemiology of Hepatitis C virus genotypes in Khyber Pakhtoonkhaw of Pakistan. *Virology Journal*, *7*(1), 203.

Ali, S., Ahmad, A., Khan, R. S., Khan, S., Hamayun, M., Khan, S. A., ... & Baig, A. H. (2014). Genotyping of HCV RNA reveals that 3a is the most prevalent genotype in Mardan, Pakistan. *Advances in Virology*, *2014*.

Ali, S., Ali, I., Azam, S., & Ahmad, B. (2011). Frequency distribution of HCV genotypes among chronic hepatitis C patients of Khyber Pakhtunkhwa. *Virology Journal*, *8*(1), 193.

Aziz, H., Raza, A., Murtaza, S., Waheed, Y., Khalid, A., Irfan, J., ... & Athar, M. A. (2013). Molecular epidemiology of hepatitis C virus genotypes in different geographical regions of Punjab Province in Pakistan and a phylogenetic analysis. *International Journal of Infectious Diseases*, *17*(4), e247-e253.

Basit, A., Rahim, K., Ahmad, I., Shafiq, M., Mushtaq, S., Shaheen, H., & Khan, I. (2014). Prevalence of hepatitis B and C infection in Pakistan. *Journal of Infection Molecular Biology*, *2*(3), 35-8.

Butt, S., Idrees, M., Akbar, H., ur Rehman, I., Awan, Z., Afzal, S., ... & Rafique, S. (2010). The changing epidemiology pattern and frequency distribution of hepatitis C virus in Pakistan. *Infection, Genetics, and Evolution*, *10*(5), 595-600.

Chen, S. L., & Morgan, T. R. (2006). The natural history of hepatitis C virus (HCV) infection. *International Journal of Medical Science*, *3*(2), 47-52.

Chevaliez, S., & Pawlotsky, J. M. (2007). Hepatitis C virus: virology, diagnosis, and management of antiviral therapy. *World Journal of gastroenterology*, *13*(17), 2461.

Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L. R., Bradley, D. W., & Houghton, M. (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non—B viral hepatitis genom e. *Science*, *244*, 359-362.

Choo, Q. L., Richman, K. H., Han, J. H., Berger, K., Lee, C., Dong, C., ... & Barr, P. J. (1991). Genetic organization and diversity of the hepatitis C virus. *Proceedings of the National Academy of Sciences*, *88*(6), 2451-2455.

Csete, J., Elliott, R., & Fischer, B. (2008). *" Viral Time Bomb": Health and Human Rights Challenges in Addressing Hepatitis C in Canada*. Centre for Addictions Research of BC.

Domingo, E., Sheldon, J., & Perales, C. (2012). Viral quasispecies evolution. *Microbiology and Molecular Biology Reviews*, *76*(2), 159-216.

Elasifer, H. A., Agnnyia, Y. M., Al-Alagi, B. A., & Daw, M. A. (2010). Epidemiological manifestations of hepatitis C virus genotypes and its association with potential risk factors among Libyan patients. *Virology journal*, *7*(1), 317.

Gower, E., Estes, C., Blach, S., Razavi-Shearer, K., & Razavi, H. (2014). Global Epidemiology and genotype distribution of the hepatitis C virus infection. *Journal of Hepatology*, *61*(1), S45-S57.

Idrees, M., & Riazuddin, S. (2008). Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. *BMC Infectious Diseases*, *8*, 69.

Ijaz, B., Ahmad, W., Javed, F. T., Gull, S., Sarwar, M. T., Kausar, H., ... & Sumrin, A. (2011). Association of laboratory parameters with viral factors in patients with hepatitis C. *Virology Journal*, *8*(1), 361.

Imran, M., Manzoor, S., Khattak, N. M., Tariq, M., Khalid, M., Javed, F., & Bhatti, S. (2014). Correlation of OAS1 gene polymorphism at exon 7 splice acceptor site with interferon-based therapy of HCV infection in Pakistan. *Viral immunology*, *27*(3), 105-111.

Inamullah, I., Idrees, M., Ahmed, H., Ali, M., Ali, L., & Ahmed, A. (2011). Hepatitis C virus genotypes circulating in district Swat of Khyber Pakhtoonkhaw, Pakistan. *Virology Journal*, *8*(1), 16.

Ju, W., Yang, S., Feng, S., Wang, Q., Liu, S., Xing, H., ... & Cheng, J. (2015). Hepatitis C virus genotype and subtype distribution in Chinese chronic hepatitis C patients: nationwide spread of HCV genotypes 3 and 6. *Virology journal*, *12*(1), 109.

Karoney, M. J., & Siika, A. M. (2013). Hepatitis C virus (HCV) infection in Africa: a review. *Pan African Medical Journal*, *14*(1).

Khan, N., Ahmed, S. M., Khalid, M. M., Siddiqui, S. H., & Merchant, A. A. (2010). Effect of gender and age on the knowledge, attitude and practice regarding hepatitis B and C and vaccination

status of hepatitis B among medical students of Karachi. *Pakistan. Journal of Pakaistan Medical Associate*, *60*(6), 450-5.

Khan, N., Akmal, M., Hayat, M., Umar, M., Ullah, A., Ahmed, I., ... & Saleha, S. (2014). Geographic distribution of hepatitis C virus genotypes in Pakistan. *Hepatitis monthly*, *14*(10).

Khan, S., Attaullah, S., Ali, I., Ayaz, S., Khan, S. N., Siraj, S., & Khan, J. (2011). Rising burden of Hepatitis C Virus in hemodialysis patients. *Virology Journal*, *8*(1), 438.

Khan, S., Attaullah, S., Ayaz, S., Khan, S. N., Shams, S., Ali, I., ... & Siraj, S. (2011). Molecular epidemiology of hcv among health care workers of Khyber Pakhtunkhwa. *Virology Journal*, *8*(1), 105.

Khan, T. M., Mehr, M. T., Ullah, H., Khan, H., & Iman, N. U. (2014). Frequency of hepatitis C virus genotypes in the north of Pakistan. *Gomal Journal of Medical Sciences*, *12*(2).

Lavanchy, D. (1999). Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *Journal of viral hepatitis*, *6*(1), 35-47

Lavanchy, D. (2011). Evolving epidemiology of hepatitis C virus. *Clinical Microbiology and Infection*, *17*(2), 107-115.

Lemoine, M., Nayagam, S., & Thursz, M. (2013). Viral hepatitis in resource-limited countries and access to antiviral therapies: current and future challenges. *Future Virology*, *8*(4), 371-380.

Liang, T. J., Rehermann, B., Seeff, L. B., & Hoofnagle, J. H. (2000). Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Annals of internal medicine*, *132*(4), 296-305.

Mahmood, K., Mohammad, N., & Abidullah, A. (2011). Genotype variation of hepatitis C virus in District Buner Swat. *Journal of Ayub Medical College Abbottabad*, *23*(4).

Messina, J. P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G. S., Pybus, O. G., & Barnes, E. (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*, *61*(1), 77-87.

Mohd Hanafiah, K., Groeger, J., Flaxman, A. D., & Wiersma, S. T. (2013). Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. *Hepatology*, *57*(4), 1333-1342.

Muhammad, N., & Jan, M. A. (2005). Frequency of hepatitis" C" in Buner, NWFP. *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP*, *15*(1), 11-14.

Nabi, S. G., Zaffar, G., Sheikh, N. I., Hassan, K., & Hassan, U. (2013). Hepatitis C virus genotypes: a plausible association with viral loads. *Indian Journal of Pathology and Microbiology*, *56*(4), 384.

Nouroz, F., Shaheen, S., Mujtaba, G., & Noreen, S. (2015). An overview of hepatitis C virus genotypes and its control. *Egyptian Journal of Medical Human Genetics*, *16*(4), 291-298.

Niu, Z., Zhang, P., & Tong, Y. (2016). Age and gender distribution of Hepatitis C virus prevalence and genotypes of individuals of physical examination in WuHan, Central China. *SpringerPlus*, *5*(1), 1557. http://doi.org/10.1186/s40064-016-3224-z

Ohno, O.,Mizokami, M., Wu, R. R., Saleh, M. G., Ohba, K. I., Orito, E., & Lau, J. Y. (1997). New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *Journal of clinical microbiology*, *35*(1), 201-207.

Owiti, J. A., Greenhalgh, T., Sweeney, L., Foster, G. R., & Bhui, K. S. (2015). Illness perceptions and explanatory models of viral hepatitis B & C among immigrants and refugees: a narrative systematic review. *BioMed Central public health*, *15*(1), 151.

Pawlotsky, J. M., Aghemo, A., Back, D., Dusheiko, G., Forns, X., Puoti, M., & Sarrazin, C. (2015). EASL recommendations on treatment of hepatitis C 2015. *Journal of Hepatology*, *63*(1), 199-236.

Perz, J. F., Thompson, N. D., Schaefer, M. K., & Patel, P. R. (2010). US outbreak investigations highlight the need for safe injection practices and basic infection control. *Clinics in liver disease*, *14*(1), 137-151.

Protzer, U., Maini, M. K., & Knolle, P. A. (2012). Living in the liver: hepatic infections. *Nature Reviews Immunology*, *12*(3), 201-213.

Petruzziello, A., Marigliano, S., Loquercio, G., Cozzolino, A., & Cacciapuoti, C. (2016). Global epidemiology of hepatitis C virus infection: an up-date of the distribution and circulation of hepatitis C virus genotypes. *World Journal of Gastroenterology*, *22*(34), 7824.

Qureshi, H., Arif, A., Ahmed, W., & Alam, S. E. (2007). HCV exposure in spouses of the index cases. *JPMA. The Journal of the Pakistan Medical Association*

Rauf, A., Nadeem, M. S., Ali, A., Iqbal, M., Mustafa, M., Muzammal Latif, M., ... & Shakoori, A. R. (2010). Prevalence of hepatitis B and C in internally displaced persons of war against terrorism in Swat, Pakistan. *The European Journal of Public Health*, *21*(5), 638-642.

Saeed, U., Waheed, Y., & Ashraf, M. (2014). Hepatitis B and hepatitis C viruses: a review of viral genomes, viral induced host immune responses, genotypic distributions, and worldwide epidemiology. *Asian Pacific Journal of Tropical Disease*, *4*(2), 88-96.

Saleha, S., Kamal, A., Ullah, F., Khan, N., Mahmood, A., & Khan, S. (2014). Prevalence of hepatitis C virus genotypes in district Bannu, Khyber Pakhtunkhwa, Pakistan. *Hepatitis esearch and treatment*, *2014*.

Smith, D. B., Bukh, J., Kuiken, C., Muerhoff, A. S., Rice, C. M., Stapleton, J. T., & Simmonds, P. (2014). Expanded Classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology*, *59*(1), 318-327.

ur Rehman, L., Ullah, I., Ali, I., Khan, I. A., Iqbal, A., Khan, S., ... & Jahangiri, A. T. (2011). Active hepatitis C infection and HCV genotypes prevalent among the IDUs of Khyber Pakhtunkhwa. *Virology Journal*, *8*(1), 327.

Waheed, Y., Shafi, T., Safi, S. Z., & Qadri, I. (2009). Hepatitis C virus in Pakistan: A systematic review of prevalence, genotypes and risk factors. *World Journal of Gastroenterology: WJG*, *15*(45), 5647–5653. http://doi.org/10.3748/wjg.15.5647

Wan, Z., Chen, Q., Chen, X., Duo, L., Li, P., Zheng, Y. T., & Zhang, C. (2016). HCV Diversity among Chinese and Burmese IDUs in Dehong, Yunnan, China. *PloS one*, *11*(9), e0163062.

Weiner, A. J., Brauer, M. J., Rosenblatt, J., Richman, K. H., Tung, J., Crawford, K., ... & Han, J. H. (1991). Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. *Virology*, *180*(2), 842-848.

Wu, T., Xiong, L., Wang, F., Xu, X., Wang, J., Lin, F., ... & Zhou, Y. (2016). A unique pattern of HCV genotype distribution on Hainan island in China revealed by evolutionary analysis. *Cellular Physiology and Biochemistry*, *39*(1), 316-330.

Yahya, K. M., Khan, R. R., Aziz, U., & Hussain, S. A. (2012). Frequency of various hepatitis C virus (HCV) genotypes in Faisalabad and surrounding districts. *Methodology*.

Zanetti, A. R. (1999). Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *Journal of viral hepatitis*, *6*(1), 35-47.

APPENDIX 1

Table: HCV samples details used in this study

Age	Gender	Labcode	Result	Region
29	Female	678	HCV 3a Genotype	Rawalpindi
45	Female	165	HCV 3a Genotype	Gujranwala
56	Female	162	HCV 3a Genotype	Islamabad
30	Female	1590	HCV 3a Genotype	Rawalpindi
62	Male	1898	HCV 3a Genotype	Gujranwala
55	Female	911	HCV 3a Genotype	Rawalpindi
32	Female	420	HCV 3a Genotype	Sawabi
50	Female	2207	HCV 3a Genotype	Sargodha
38	Male	3272	HCV 3a Genotype	Rawalpindi
33	Female	896	Undetectable Genotype	Gujranwala
40	Male	1035	HCV 3a Genotype	Attock
37	Female	955	HCV 3a,3b Mix Genotype	Rawalpindi
35	Female	2479	HCV 3a Genotype	Rawalpindi
26	Male	3554	HCV 3a Genotype	Islamabad
40	Male	147	HCV 3a Genotype	Faisalabad
60	Male	1835	HCV 3a Genotype	Peshawar
52	Female	501	HCV untypable Genotype	Jehlum
45	Female	320	HCV 3a Genotype	Faisalabad
23	Male	2231	HCV 3a Genotype	Rawalpindi
60	Male	39	HCV 3b Genotype	Jehlum
30	Female	3695	HCV 3a Genotype	Jehlum
41	Female	577	HCV 3a Genotype	Rawalpindi
33	Male	3572	HCV 3a,3b Mix Genotype	Sawabi
37	Male	785	HCV 3a Genotype	Faisalabad
48	Male	2628	HCV 3a,3b Mix Genotype	Rawalpindi
54	Male	1371	Undetectable Genotype	Jehlum
45	Male	2540	HCV 3b Genotype	Faisalabad
45	Male	1	HCV 3a Genotype	Islamabad
55	Female	1625	HCV 3a Genotype	Gujranwala
40	Male	2742	HCV 3a Genotype	Mardan
51	Male	3027	HCV 3a Genotype	Jhang
29	Female	678	HCV 3a Genotype	Rawalpindi
45	Female	165	HCV 3a Genotype	Gujranwala

18	Male	2073	HCV 3a Genotype	Rawalpindi
29	Female	2954	HCV 3a Genotype	Gujranwala
78	Female	3067	HCV 3a Genotype	Jehlum
28	Female	566	HCV 3a Genotype	Faisalabad
50	Female	3171	HCV 3a Genotype	Islamabad
43	Female	190	HCV 3a Genotype	Gujranwala
52	Male	990	HCV untypable Genotype	Faisalabad
40	Female	2641	HCV 3a Genotype	Multan
52	Female	3461	HCV 3a Genotype	Gujranwala
50	Female	2476	HCV 3a Genotype	Rawalpindi
35	Male	399	Undetectable Genotype	Rawalpindi
42	Male	1593	HCV 3a Genotype	Rawalpindi
23	Male	2231	HCV 3a Genotype	Sargodha
35	Male	3751	HCV 3a Genotype	Faisalabad
50	Male	348	HCV 3a Genotype	Islamabad
50	Male	149	HCV 3a Genotype	Faisalabad
55	Female	1760	Undetectable Genotype	Islamabad
70	Female	2514	Undetectable Genotype	Rawalpindi
39	Male	311	HCV 3b Genotype	Sargodha
28	Female	487	HCV 3a Genotype	Faisalabad
29	Male	1669	HCV 3a Genotype	Rawalpindi
36	Male	1576	HCV 3a Genotype	Gujranwala
56	Male	3238	HCV 3a Genotype	Sawabi
29	Male	123	HCV 3a Genotype	Faisalabad
48	Female	1187	HCV 3a Genotype	Sargodha
36	Male	2699	HCV untypable Genotype	Islamabad
45	Female	347	HCV 2a Genotype	Islamabad
42	Male	3248	HCV 3a Genotype	Faisalabad
59	Female	1205	HCV 3a Genotype	Faisalabad
60	Female	3454	HCV 2a Genotype	Jehlum
29	Female	217	HCV 3a Genotype	Rawalpindi
40	Female	37	HCV 3a Genotype	Rawalpindi
42	Male	4140	Undetectable Genotype	Gujranwala
45	Female	2520	HCV 3a Genotype	Faisalabad
45	Male	2879	HCV 3a Genotype	Gujranwala
70	Male	419	HCV 3a,3b Mix Genotype	Rawalpindi
36	Female	612	HCV 3a,3b Mix Genotype	Jhang
28	Female	820	HCV 3a Genotype	Gujranwala
45	Female	1769	HCV 3a Genotype	Rawalpindi

60	Female	833	Undetectable Genotype	Rawalpindi
50	Female	218	HCV untypable Genotype	Jehlum
17	Female	539	HCV 3a Genotype	Rawalpindi
47	Female	2041	HCV 3a Genotype	Gujranwala
80	Male	859	HCV 3a,3b Mix Genotype	Faisalabad
60	Female	1180	HCV 3b Genotype	Gujranwala
36	Female	1170	Undetectable Genotype	Rawalpindi
31	Male	806	HCV 3a Genotype	Sargodha
48	Female	2512	HCV 3a Genotype	Gujranwala
40	Male	320	HCV 3a Genotype	Gujranwala
60	Male	1762	HCV 3a Genotype	Islamabad
40	Male	892	HCV 3b Genotype	Islamabad
47	Female	2066	HCV 4 Genotype	Faisalabad
60	Female	3743	HCV 3a,3b Mix Genotype	Faisalabad
41	Female	401	HCV 3a Genotype	Sargodha
50	Male	963	HCV 3b Genotype	Faisalabad
33	Female	2697	HCV 3a Genotype	Rawalpindi
47	Male	2194	HCV 3a,3b Mix Genotype	Faisalabad
43	Female	2608	HCV 3a Genotype	Faisalabad
35	Female	3406	HCV 3a Genotype	Faisalabad
40	Female	3064	Undetectable Genotype	Gujranwala
47	Male	2189	HCV 3a Genotype	Gujranwala
42	Female	125	HCV 3a Genotype	Faisalabad
60	Male	1073	HCV 3a Genotype	Jehlum
44	Female	1922	HCV 3a Genotype	Jehlum
56	Female	1739	HCV 3a Genotype	Gujranwala
33	Female	2308	HCV 3a Genotype	Jehlum
28	Female	372	HCV 2a Genotype	Sawabi
48	Male	172	HCV 3a Genotype	Gujranwala
45	Male	38	HCV 3a Genotype	Jehlum
49	Female	1055	HCV 3a Genotype	Sargodha
46	Male	2610	HCV 3a Genotype	Gujranwala
68	Male	694	HCV 3a Genotype	Islamabad
56	Male	907	HCV 3a Genotype	Islamabad
24	Female	4232	HCV 3a Genotype	Rawalpindi
55	Male	2537	HCV 2a Genotype	Sargodha
51	Male	1230	HCV 3b Genotype	Sawabi
25	Male	1504	HCV 3a Genotype	Islamabad
51	Male	323	HCV 3a Genotype	Rawalpindi

65	Female	1196	HCV 3a Genotype	Islamabad
45	Female	732	HCV 3a Genotype	Faisalabad
35	Male	534	HCV 3a Genotype	Gujranwala
42	Female	782	HCV 3a Genotype	Gujranwala
45	Female	231	HCV 3a Genotype	Gujranwala
72	Female	512	HCV 3a Genotype	Gujranwala
52	Male	2601	HCV 3a Genotype	Rawalpindi
51	Male	3517	HCV 3a Genotype	Islamabad
60	Male	404	HCV 3a,3b Mix Genotype	Gujranwala
32	Female	2688	HCV 3a Genotype	Islamabad
28	Female	3068	HCV 3b Genotype	Hangu
50	Male	3187	HCV 3a Genotype	Rawalpindi
36	Male	19	HCV 3a Genotype	Faisalabad
49	Male	1185	HCV 3a Genotype	Sargodha
52	Male	963	HCV 3a Genotype	Gujranwala
35	Male	2931	HCV 3a Genotype	Mardan
26	Female	48	HCV 3a Genotype	Faisalabad
50	Male	633	HCV 3a Genotype	Rawalpindi
50	Male	149	HCV 3a Genotype	Faisalabad
40	Male	445	HCV 3a Genotype	Jehlum
70	Female	342	HCV 3a,3b Mix Genotype	Peshawar
46	Male	1195	HCV 3a Genotype	Gujranwala
70	Female	1078	HCV 3b Genotype	Jehlum
19	Female	1316	HCV 3a Genotype	Faisalabad
40	Female	2222	Undetectable Genotype	Jehlum
39	Male	352	HCV 3a Genotype	Rawalpindi
31	Female	2707	HCV 3a Genotype	Islamabad
30	Male	3462	HCV 3a Genotype	Gujranwala
32	Female	455	HCV 3a Genotype	Jehlum
34	Female	1636	HCV 3a Genotype	Jhang
37	Male	2781	HCV 3a Genotype	Faisalabad
37	Female	27	Undetectable Genotype	Sargodha
42	Female	1737	HCV 3a Genotype	Gujranwala
23	Female	3555	HCV 3a Genotype	Islamabad
60	Female	350	HCV 3a Genotype	Faisalabad
25	Female	2896	HCV 3a Genotype	Faisalabad
63	Male	359	HCV 3b Genotype	Islamabad
30	Male	2733	HCV 3a Genotype	Rawalpindi
40	Female	41	HCV 2a Genotype	Rawalpindi

45	Male	580	HCV 3a Genotype	Jhang
70	Female	333	HCV 3a Genotype	Rawalpindi
21	Male	4153	HCV 3a Genotype	Faisalabad
41	Male	117	HCV 3a Genotype	Gujranwala
48	Female	1721	HCV 3a Genotype	Jehlum
52	Female	501	HCV untypable Genotype	Jehlum
40	Female	41	HCV 2a Genotype	Rawalpindi
50	Female	2466	HCV 3a Genotype	Faisalabad
66	Female	507	HCV 3a Genotype	Jehlum
68	Male	694	HCV 3a Genotype	Islamabad
30	Male	158	HCV 3a Genotype	Peshawar
41	Male	218	Undetectable Genotype	Faisalabad
49	Male	190	HCV 3a Genotype	Islamabad
36	Female	475 /	HCV 3a Genotype	Gujranwala
60	Female	962	HCV 3a Genotype	Gujranwala
22	Female	3129	HCV 3a Genotype	Jehlum
55	Female	1610	HCV 3a Genotype	Mardan
40	Male	3261	HCV 3a Genotype	Faisalabad
72	Male	161	HCV 3a Genotype	Islamabad
45	Female	827	HCV untypable Genotype	Islamabad
45	Female	2759	HCV 3a Genotype	Faisalabad
45	Female	101	HCV 3a Genotype	Faisalabad
40	Female	3636	HCV 3b Genotype	Gujranwala
40	Female	1135	HCV 3a Genotype	Jehlum
70	Male	2440	HCV 3a Genotype	Gujranwala
40	Female	3725	Undetectable Genotype	Gujranwala
60	Female	469	HCV 3a,3b Mix Genotype	Islamabad
52	Female	3156	HCV 3a Genotype	Rawalpindi
57	Female	2663	HCV 3b Genotype	Sawabi
48	Male	3599	HCV 3a,3b Mix Genotype	Islamabad
39	Male	31	HCV 3a Genotype	Sargodha
50	Male	815	HCV 3a Genotype	Faisalabad
33	Female	1586	HCV 2a Genotype	Islamabad
46	Male	808	HCV 3a Genotype	Sargodha
30	Female	199	HCV 3a Genotype	Islamabad
42	Female	3500	HCV 3b Genotype	Jehlum
25	Female	2755	HCV 3a Genotype	Faisalabad
45	Female	458	HCV 3a Genotype	Jehlum
50	Male	563	HCV 3a Genotype	Gujranwala

36	Female	612	HCV 3a,3b Mix Genotype	Jhang
24	Female	1464	HCV 2a Genotype	Sawabi
49	Female	2603	HCV 3a Genotype	Faisalabad
56	Female	228	HCV 3a Genotype	Islamabad
45	Female	54	HCV 3b Genotype	Mardan
55	Male	898	HCV 3a Genotype	Rawalpindi
36	Female	2439	HCV 3a Genotype	Rawalpindi
64	Male	3045	HCV 3a Genotype	Rawalpindi
66	Male	2975	HCV untypable Genotype	Islamabad
55	Female	3065	HCV 3a Genotype	Jehlum
56	Female	912	HCV 3a Genotype	Rawalpindi
57	Female	1132	HCV 3a Genotype	Jehlum
48	Male	801	HCV 3a Genotype	Islamabad
64	Female	668	HCV 3a Genotype	Multan
43	Male	8	HCV 3a Genotype	Faisalabad
56	Female	2801	HCV 3a Genotype	Sawabi
30	Female	1233	HCV untypable Genotype	Faisalabad
45	Female	3410	HCV 3a,3b Mix Genotype	Faisalabad
60	Female	1053	HCV 3a Genotype	Mardan
66	Male	343	HCV 3a Genotype	Islamabad
35	Female	210	HCV 3a Genotype	Jehlum
55	Female	2414	HCV 3a Genotype	Rawalpindi
52	Female	3084	Undetectable Genotype	Rawalpindi
50	Male	1208	HCV 3a Genotype	Rawalpindi
37	Male	2573	HCV 3a Genotype	Jehlum
58	Female	1283	HCV 3a Genotype	Faisalabad
48	Female	2955	HCV 3a Genotype	Gujranwala
50	Female	3091	HCV 3a Genotype	Faisalabad
50	Male	2731	HCV 3a,3b Mix Genotype	Rawalpindi
20	Female	2035	HCV 3a Genotype	Islamabad
40	Male	3584	HCV 3a Genotype	Gujranwala
25	Male	2841	HCV 3a Genotype	Jehlum
45	Female	617	HCV untypable Genotype	Rawalpindi
35	Female	1352	HCV 3b Genotype	Rawalpindi
68	Female	1173	HCV 3a Genotype	Rawalpindi
55	Female	25	HCV 3a,3b Mix Genotype	Gujranwala
55	Male	3460	HCV 3a Genotype	Gujranwala
27	Female	56	HCV 3a Genotype	Jehlum
30	Female	1367	HCV 3a Genotype	Jehlum

46	Female	323	HCV 3a Genotype	Rawalpindi
75	Female	1244	HCV 3a,3b Mix Genotype	Jehlum
42	Female	2433	HCV 3a Genotype	Rawalpindi
35	Male	2188	HCV 3a Genotype	Gujranwala
45	Male	2133	HCV 3a Genotype	Faisalabad
60	Female	1091	HCV 3a Genotype	Rawalpindi
50	Male	814	HCV 3a,3b Mix Genotype	Peshawar
45	Male	270	HCV untypable Genotype	Hangu
40	Female	224	HCV 3a Genotype	Rawalpindi
42	Female	125	HCV 3a Genotype	Faisalabad
40	Female	1020	HCV 3a Genotype	Islamabad
30	Male	513	HCV 3a Genotype	Gujranwala
42	Male	2536	HCV 3a,3b Mix Genotype	Sargodha
60	Male	404	HCV 3a,3b Mix Genotype	Gujranwala
25	Female	2940	HCV 3a Genotype	Rawalpindi
50	Male	1709	Undetectable Genotype	Rawalpindi
30	Female	3655	HCV 3a Genotype	Faisalabad
50	Female	3717	HCV 3a Genotype	Gujranwala
18	Male	465	HCV 3a Genotype	Rawalpindi
38	Male	1174	HCV 3a Genotype	Rawalpindi
60	Female	14	HCV untypable Genotype	Gujranwala
75	Male	568	HCV 3a Genotype	Rawalpindi
42	Female	1734	HCV 3a Genotype	Rawalpindi
45	Male	38	HCV 3a Genotype	Jehlum
23	Male	2569	HCV 3a Genotype	Islamabad
45	Female	732	HCV 3a Genotype	Faisalabad
60	Male	367	HCV 3a Genotype	Gujranwala
45	Female	63	HCV 3a Genotype	Faisalabad
70	Female	1240	HCV 3a Genotype	Islamabad
40	Female	248	HCV 3a Genotype	Peshawar
55	Female	212	HCV 3a Genotype	Jehlum
44	Female	321	HCV 3a Genotype	Sargodha
38	Female	1079	HCV 3a Genotype	Jehlum
51	Female	1187	Undetectable Genotype	Faisalabad
15	Female	1701	HCV 3a Genotype	Faisalabad
45	Female	827	HCV untypable Genotype	Islamabad
26	Female	990	HCV 3a Genotype	Peshawar
47	Female	2936	HCV 3a,3b Mix Genotype	Faisalabad
62	Female	212	HCV 3a Genotype	Gujranwala

28	Male	2150	HCV 3a Genotype	Faisalabad
23	Male	3102	HCV 3a Genotype	Rawalpindi
30	Male	772	HCV 3a Genotype	Rawalpindi
39	Male	154	HCV 3b Genotype	Rawalpindi
36	Female	4144	HCV 3a,3b Mix Genotype	Islamabad
43	Male	841	HCV 3a Genotype	Gujranwala
20	Female	3569	HCV 3a Genotype	Gujranwala
36	Female	4026	HCV 3a Genotype	Attock
13	Female	1035	HCV untypable Genotype	Peshawar
28	Male	2687	HCV 3a Genotype	Jehlum
60	Female	3951	HCV 3a,3b Mix Genotype	Faisalabad
30	Female	3093	HCV 3a Genotype	Faisalabad
23	Male	2310	HCV 3a Genotype	Rawalpindi
24	Female	2438	HCV untypable Genotype	Islamabad
53	Male	3393	HCV 3a Genotype	Rawalpindi
45	Female	235	HCV 3a,3b Mix Genotype	Gujranwala
42	Male	1167	HCV 3b Genotype	Islamabad
70	Male	2301	HCV 3a,3b Mix Genotype	Islamabad
38	Male	3241	HCV 3a Genotype	Faisalabad
38	Female	4236	HCV 3a Genotype	Rawalpindi
44	Male	2650	HCV 3a Genotype	Rawalpindi
55	Female	962	HCV 3a Genotype	Faisalabad
39	Male	154	HCV 3b Genotype	Rawalpindi
32	Male	3445	HCV 3a Genotype	Sargodha
40	Female	37	HCV 3a Genotype	Rawalpindi
45	Female	1223	HCV 3a Genotype	Jehlum
50	Female	309	HCV 3a Genotype	Faisalabad
51	Male		HCV 3a Genotype	Jhang
31	Female	1975	HCV 3a Genotype	Faisalabad
32	Male	1227	HCV 3a Genotype	Jehlum
43	Female	3082	HCV 3a Genotype	Islamabad
35	Male	2831	HCV 3a,3b Mix Genotype	Faisalabad
55	Female	1225	HCV 3b Genotype	Rawalpindi
36	Female	918	HCV 3a Genotype	Sawabi
65	Male	1383	HCV 3a Genotype	Rawalpindi
30	Female	3173	HCV 3a Genotype	Islamabad
48	Male	2086	HCV 3a Genotype	Islamabad
31	Female	428	HCV 3a Genotype	Islamabad
37	Male	172	HCV 3a Genotype	Sargodha

71	Male	36	HCV 3a Genotype	Islamabad
40	Male	147	HCV 3a Genotype	Faisalabad
65	Female	24	HCV 3a Genotype	Gujranwala
43	Male	8	HCV 3a Genotype	Faisalabad
35	Female	218	HCV 3a Genotype	Rawalpindi
28	Female	249	HCV 3a Genotype	Rawalpindi
40	Female	128	Undetectable Genotype	Faisalabad
70	Female	818	HCV untypable Genotype	Gujranwala
40	Female	1671	HCV 3a Genotype	Rawalpindi
44	Female	2757	HCV 3a Genotype	Jehlum
40	Female	3144	HCV 3b Genotype	Sawabi
51	Male	497	HCV 3a Genotype	Rawalpindi
25	Male	947	HCV 3b Genotype	Faisalabad
25	Female	636	HCV 3a Genotype	Faisalabad
42	Male	2536	HCV 3a,3b Mix Genotype	Sargodha
60	Female	705	HCV 3a Genotype	Islamabad
38	Male	3428	HCV 3a Genotype	Sawabi
55	Female	2892	HCV 3a Genotype	Rawalpindi
42	Female	1645	HCV 3a Genotype	Jehlum
60	Female	96	HCV 3a Genotype	Rawalpindi
32	Female	:2688	HCV 3a Genotype	Islamabad
30	Male	1857	HCV 3a Genotype	Faisalabad
42	Female	814	Undetectable Genotype	Gujranwala
37	Female	2077	HCV 2a Genotype	Jehlum
50	Male	963	HCV 3b Genotype	Faisalabad
40	Female	1299	HCV 3a Genotype	Rawalpindi
50	Male	563	HCV 3a Genotype	Gujranwala
72	Male	153	HCV 3a Genotype	Islamabad
39	Male	2917	HCV 3a Genotype	Faisalabad
27	Female	56	HCV 3a Genotype	Jehlum
30	Female	3196	HCV 3a Genotype	Faisalabad
45	Male	1208	HCV 3a Genotype	Rawalpindi
45	Female	2299	HCV 3b Genotype	Rawalpindi
66	Female	3170	HCV 3a Genotype	Rawalpindi
26	Female	34	HCV 3a Genotype	Faisalabad
50	Female	1369	HCV 3a Genotype	Jehlum
65	Female	1438	HCV 3a Genotype	Rawalpindi
20	Male	163	HCV 3b Genotype	Islamabad
30	Female	3740	HCV 3a Genotype	Faisalabad

45	Female	320	HCV 3a Genotype	Faisalabad
36	Female	3538	HCV 3a Genotype	Rawalpindi
45	Female	2004	HCV 3a Genotype	Faisalabad
34	Female	678	HCV 3a Genotype	Rawalpindi
47	Male	41	HCV 3a Genotype	Sargodha
22	Male	1166	HCV 3a Genotype	Faisalabad
17	Male	877	HCV 3a Genotype	Faisalabad
45	Male	794	HCV 3a Genotype	Gujranwala
28	Male	346	HCV untypable Genotype	Islamabad
75	Male	568	HCV 3a Genotype	Rawalpindi
40	Male	1697	HCV 3a Genotype	Faisalabad
35	Female	906	HCV 3a Genotype	Gujranwala
50	Female	3000	HCV 3a Genotype	Gujranwala
45	Female	164	HCV 3a Genotype	Gujranwala
48	Female	2042	HCV 3a Genotype	Gujranwala
60	Female	833	Undetectable Genotype	Rawalpindi
54	Female	1254	HCV 3a Genotype	Rawalpindi
40	Female	126	HCV untypable Genotype	Faisalabad
40	Female	1331	HCV 3a Genotype	Gujranwala
45	Female	3869	HCV 3a Genotype	Sargodha
66	Female	507	HCV 3a Genotype	Jehlum
40	Male	3413	HCV 3a Genotype	Faisalabad
50	Female	2083	HCV 3a Genotype	Jehlum
44	Female	214	HCV 3a Genotype	Jehlum
65	Male	2190	HCV 3a Genotype	Gujranwala
42	Female	4268	HCV 3a Genotype	Jehlum
36	Male	4269	HCV 3a Genotype	Jehlum
70	Female	2	HCV 3a,3b Mix Genotype	Islamabad
40	Male	817	HCV 3a Genotype	Peshawar
25	Female	1479	HCV 3a Genotype	Sargodha
35	Female	3064	HCV 3a,3b Mix Genotype	Hangu
25	Female	3654	HCV 3a Genotype	Faisalabad
40	Male	4060	HCV 3a Genotype	Rawalpindi
35	Female	1176	HCV 3a Genotype	Rawalpindi
25	Female	1479	HCV 3a Genotype	Sargodha
66	Male	2965	HCV untypable Genotype	Islamabad
31	Female	2544	Undetectable Genotype	Mardan
40	Female	3725	Undetectable Genotype	Gujranwala
39	Male	154	HCV 3b Genotype	Rawalpindi

50	Male	2515	HCV 3a Genotype	Rawalpindi
40	Male	1627	Undetectable Genotype	Peshawar
76	Male	2044	HCV 3a Genotype	Gujranwala
30	Male	626	HCV 3a Genotype	Faisalabad
48	Female	1940	HCV 3a Genotype	Islamabad
50	Female	939	Undetectable Genotype	Islamabad
32	Female	1022	HCV 3a Genotype	Gujranwala
46	Male	3677	HCV 3a Genotype	Gujranwala
50	Female	3963	HCV 3a Genotype	Gujranwala
51	Male	3397	HCV 3a,3b Mix Genotype	Islamabad
60	Female	1641	HCV 3a,3b Mix Genotype	Rawalpindi
40	Female	2595	HCV 3a Genotype	Jehlum
38	Female	156	HCV untypable Genotype	Islamabad
60	Male	155	HCV 3a Genotype	Islamabad
45	Female	1380	HCV 3a Genotype	Rawalpindi
40	Female	128	Undetectable Genotype	Faisalabad
55	Female	2304	HCV 3a Genotype	Jehlum
43	Female	1068	HCV 3a Genotype	Gujranwala
30	Male	2228	HCV 3a,3b Mix Genotype	Faisalabad
34	Male	3711	HCV untypable Genotype	Gujranwala
40	Male	2911	HCV 3a Genotype	Islamabad
30	Male	55	HCV 3a Genotype	Jehlum
42	Male	320	HCV 3a Genotype	Gujranwala
40	Female	892	HCV 3b Genotype	Islamabad
39	Male	3667	HCV 3a Genotype	Mardan
45	Female	2209	HCV 3a Genotype	Sargodha
64	Male	4121	HCV 3a,3b Mix Genotype	Islamabad
56	Female	2838	HCV 3a Genotype	Islamabad
30	Female	689	HCV 3a Genotype	Jehlum
60	Male	39	HCV 3b Genotype	Jehlum
63	Male	3660	HCV 3a,3b Mix Genotype	Rawalpindi
42	Female	2619	HCV 3a Genotype	Gujranwala
26	Male	2907	HCV 3a Genotype	Faisalabad
46	Female	2224	HCV 3a Genotype	Jehlum
23	Male	1873	HCV 3a Genotype	Islamabad
33	Male	3242	HCV 3a Genotype	Faisalabad
36	Male	3646	Undetectable Genotype	Rawalpindi
29	Male	2706	HCV 3a Genotype	Islamabad
45	Female	54	HCV 3b Genotype	Mardan

35	Female	2370	HCV 3a Genotype	Faisalabad
35	Female	716	HCV 3a Genotype	Rawalpindi
54	Female	1330	HCV 3a Genotype	Gujranwala
30	Female	506	HCV 3a Genotype	Gujranwala
23	Female	27	Undetectable Genotype	Sargodha
36	Male	996	HCV 3a Genotype	Faisalabad
50	Male	2810	HCV 3a Genotype	Jehlum
32	Male	3096	HCV 3a Genotype	Faisalabad
54	Female	291	HCV 3a Genotype	Jehlum
36	Male	1077	HCV 3a Genotype	Rawalpindi
58	Male	105	HCV 3a Genotype	Jehlum
25	Female	636	HCV 3a Genotype	Faisalabad
33	Male	1140	HCV 3a Genotype	Faisalabad
50	Male	907	Undetectable Genotype	Faisalabad
40	Male	1627	Undetectable Genotype	Peshawar
40	Male	3449	HCV 3b Genotype	Attock
24	Female	278	HCV 3a Genotype	Faisalabad
50	Male	571	HCV 3a Genotype	Rawalpindi
50	Female	3057	HCV 2a Genotype	Faisalabad
50	Male	3279	HCV 3a Genotype	Faisalabad
42	Female	4073	HCV 3a Genotype	Rawalpindi
55	Female	3909	HCV 3a Genotype	Jehlum
29	Female	1680	HCV 3a Genotype	Rawalpindi
48	Male	1729	HCV 3a Genotype	Islamabad
57	Male	3744	HCV untypable Genotype	Rawalpindi
20	Female	188	HCV untypable Genotype	Islamabad
30	Male	626	HCV 3a Genotype	Faisalabad
45	Male	2973	HCV 3a Genotype	Islamabad
50	Male	384	HCV 3a Genotype	Gujranwala
50	Male	571	HCV 3a Genotype	Rawalpindi
35	Female	3035	HCV 3a,3b Mix Genotype	Jehlum
22	Male	437	HCV 3a Genotype	Jehlum
35	Female	3141	HCV 3a Genotype	Gujranwala
17	Male	2933	HCV 3a Genotype	Mardan
34	Male	392	HCV 3a,3b Mix Genotype	Mardan
68	Female	3066	HCV 3a Genotype	Jehlum
37	Female	47	HCV 3a Genotype	Islamabad
50	Male	3094	HCV 3a Genotype	Faisalabad
45	Female	2541	HCV 3a Genotype	Gujranwala

40	Female	126	HCV untypable Genotype	Faisalabad
30	Male	513	HCV 3a Genotype	Gujranwala
60	Female	350	HCV 3a Genotype	Faisalabad
48	Male	1267	HCV 3a Genotype	Faisalabad
39	Male	2878	HCV 3a Genotype	Gujranwala
48	Male	2932	HCV 3a,3b Mix Genotype	Mardan
35	Female	2124	HCV 3a Genotype	Gujranwala
59	Male	2542	HCV 3a Genotype	Gujranwala
46	Female	2568	HCV 3b Genotype	Islamabad
37	Female	482	HCV 3a,3b Mix Genotype	Islamabad
38	Female	599	Undetectable Genotype	Sawabi
50	Female	1230	HCV 3a Genotype	Faisalabad
64	Male	4145	HCV 3a Genotype	Islamabad
45	Male	3047	Undetectable Genotype	Islamabad
40	Male	1241	HCV 3a Genotype	Rawalpindi
65	Female	2141	HCV 3a Genotype	Jhang
33	Male	1384	HCV 3a Genotype	Rawalpindi
40	Male	4270	HCV 3a Genotype	Jehlum
37	Female	2751	HCV 3a Genotype	Jehlum
49	Female	3395	HCV 3a Genotype	Faisalabad
30	Male	4139	HCV 3a Genotype	Gujranwala
70	Female	809	HCV 3a Genotype	Sargodha
60	Male	404	HCV 3a,3b Mix Genotype	Gujranwala
27	Male	1743	HCV 3a,3b Mix Genotype	Islamabad
45	Female	3522	HCV 3a Genotype	Gujranwala
52	Female	204	HCV 3a Genotype	Rawalpindi
11	Male	4010	HCV 3a,3b Mix Genotype	Gujranwala
45	Female	320	HCV 3a Genotype	Faisalabad
39	Female	1948	HCV 3a Genotype	Rawalpindi
45	Male	2629	HCV 3a Genotype	Faisalabad
43	Male	272	HCV 3a Genotype	Faisalabad
45	Female	2478	HCV 3a Genotype	Faisalabad
37	Female	2043	HCV 3a Genotype	Sawabi
30	Male	2698	HCV 3a Genotype	Rawalpindi
24	Female	471	HCV 3a,3b Mix Genotype	Gujranwala
70	Female	104	HCV untypable Genotype	Rawalpindi
30	Male	1692	HCV untypable Genotype	Faisalabad
50	Female	2979	HCV 3a Genotype	Jehlum
29	Male	123	HCV 3a Genotype	Faisalabad

40	Male	3162	HCV 3a Genotype	Islamabad
46	Female	516	HCV 3a Genotype	Sargodha
50	Female	2678	HCV 3a Genotype	Gujranwala
48	Male	561	HCV 3a Genotype	Faisalabad
27	Female	2696	HCV 3a Genotype	Sargodha
25	Male	45	HCV 3b Genotype	Islamabad
35	Male	1171	HCV 3a Genotype	Rawalpindi
27	Male	372	HCV 3a,3b Mix Genotype	Faisalabad
60	Female	452	HCV 3a Genotype	Jehlum
45	Female	307	HCV 3a Genotype	Gujranwala
37	Female	2751	HCV 3a Genotype	Jehlum
48	Female	88	HCV 3a Genotype	Rawalpindi
45	Male	532	HCV 3a Genotype	Gujranwala
60	Male	2135	HCV 3a Genotype	Gujranwala
44	Male	214	HCV 3a Genotype	Jehlum
30	Male	3415	HCV 2a Genotype	Faisalabad
35	Male	298	HCV 3a Genotype	Peshawar
25	Male	947	HCV 3b Genotype	Faisalabad
36	Female	1573	HCV 3a Genotype	Gujranwala
50	Female	1720	HCV 3a,3b Mix Genotype	Rawalpindi
36	Female	3746	HCV 3a,3b Mix Genotype	Rawalpindi
24	Female	3053	HCV 3a Genotype	Rawalpindi
45	Female	651	HCV 3a Genotype	Faisalabad
35	Male	3071	HCV 3a Genotype	Faisalabad
35	Female	1210	HCV 2a Genotype	Gujranwala
30	Male	55	HCV 3a Genotype	Jehlum
37	Male	3668	HCV 3a Genotype	Mardan
37	Female	47	HCV 3a Genotype	Islamabad
38	Male	2221	HCV 3a Genotype	Rawalpindi
29	Male	3063	HCV 3a Genotype	Rawalpindi
52	Male	2784	HCV 3a Genotype	Gujranwala
48	Female	613	HCV 3a,3b Mix Genotype	Jehlum
61	Female	1694	HCV 3a,3b Mix Genotype	Faisalabad
37	Female	608	HCV untypable Genotype	Jehlum
56	Female	228	HCV 3a Genotype	Islamabad
32	Female	346	HCV 3a Genotype	Attock
60	Female	1643	HCV 3a Genotype	Jehlum
65	Female	24	HCV 3a Genotype	Gujranwala
56	Female	228	HCV 3a Genotype	Islamabad

60	Female	423	HCV 3a Genotype	Sawabi
25	Female	2964	HCV 3a,3b Mix Genotype	Faisalabad
49	Female	3395	HCV 3a Genotype	Faisalabad
40	Female	2587	HCV 3a Genotype	Gujranwala
34	Female	677	HCV 3a Genotype	Gujranwala
55	Male	2654	HCV 3a Genotype	Faisalabad
30	Female	3729	HCV 3a Genotype	Islamabad
35	Male	1257	HCV 3a Genotype	Rawalpindi
66	Male	3259	Undetectable Genotype	Rawalpindi
50	Female	1239	Undetectable Genotype	Rawalpindi
50	Female	2752	HCV untypable Genotype	Jehlum
35	Male	534	HCV 3a Genotype	Rawalpindi
48	Male	1353	HCV 2a Genotype	Rawalpindi
32	Male	1766	HCV 3a Genotype	Islamabad
50	Female	1182	HCV 3a Genotype	Sargodha
55	Male	4143	HCV 3a Genotype	Islamabad
23	Female	324	HCV 3a Genotype	Rawalpindi
31	Female	2098	HCV untypable Genotype	Gujranwala
20	Male	3736	HCV 3a Genotype	Faisalabad
65	Female	1787	HCV 3a Genotype	Jehlum
42	Female	782	HCV 3a Genotype	Gujranwala
40	Male	1164	HCV 3a Genotype	Faisalabad
55	Female	3227	HCV 3a Genotype	Gujranwala
55	Male	299	HCV untypable Genotype	Rawalpindi
46	Male	259	HCV 3b Genotype	Gujranwala
30	Female	229	HCV 3a Genotype	Faisalabad
42	Female	200	HCV 3a Genotype	Islamabad
67	Female	199	HCV untypable Genotype	Islamabad
55	Male	198	HCV 3a Genotype	Islamabad
55	Female	179	HCV 3a Genotype	Peshawar
21	Male	98	HCV 3a Genotype	Faisalabad
65	Female	58	Undetectable Genotype	Islamabad
35	Male	43	HCV 3a Genotype	Rawalpindi
65	Female	42	HCV 3a Genotype	Rawalpindi
34	Male	36	HCV 3a Genotype	Rawalpindi
47	Male	4129	HCV 3a Genotype	Islamabad
32	Female	4128	HCV 3a Genotype	Islamabad
41	Female	4055	HCV 3a Genotype	Gujranwala
32	Female	3954	HCV 3a Genotype	Faisalabad

30	Female	3953	Undetectable Genotype	Faisalabad
48	Male	3929	HCV untypable Genotype	Islamabad
57	Male	3900	HCV 3a Genotype	Rawalpindi
32	Female	3877	Undetectable Genotype	Jehlum
50	Female	3749	HCV 3a Genotype	Faisalabad
60	Female	3683	HCV 3a,3b Mix Genotype	Islamabad
70	Female	3875	HCV 3a Genotype	Jehlum
70	Male	3873	HCV 3a Genotype	Jehlum
39	Female	3853	HCV 3a Genotype	Islamabad
39	Male	3652	HCV 3a Genotype	Rawalpindi
32	Male	3619	HCV 3a Genotype	Islamabad
30	Female	3593	HCV 3a Genotype	Islamabad
50	Female	3522	HCV 3a Genotype	Gujranwala
23	Male	3515	HCV 3a Genotype	Faisalabad
45	Female	3498	HCV 3a Genotype	Gujranwala
31	Male	3457	HCV 3a Genotype	Jehlum
55	Male	3442	HCV 3a Genotype	Rawalpindi
60	Female	3437	HCV 3a,3b Mix Genotype	Rawalpindi
60	Female	3431	HCV 3a Genotype	Rawalpindi
31	Male	3428	HCV 3a Genotype	Sawabi
30	Male	2211	HCV 3a Genotype	Rawalpindi
65	Male	2709	HCV 3a Genotype	Rawalpindi
60	Female	2073	HCV 3a Genotype	Rawalpindi
32	Female	2067	HCV 3a Genotype	Rawalpindi
60	Male	2074	HCV 3a Genotype	Rawalpindi
50	Female	1950	HCV 3a Genotype	Gujranwala
30	Female	2072	HCV 3a Genotype	Rawalpindi
45	Female	2238	HCV 3a,3b Mix Genotype	Rawalpindi
50	Female	2307	HCV 3a Genotype	Faisalabad
54	Female	2244	HCV 3b Genotype	Jehlum
45	Female	2272	HCV 3a Genotype	Jehlum