



Original Article

Molecular evolution, virology and spatial distribution of HCV genotypes in Pakistan: A meta-analysis

Arslan Habib^{a,*}, Nadiya Habib^b, Khalid Mahmood Anjum^c, Riffat Iqbal^d, Zeeshan Ashraf^e, Muhammad Usman Taj^e, Muhammad Asim^f, Kanwal Javid^g, Faezeh Idoon^h, Saeid Dashtiⁱ, Cassio Rocha Medeiros^j, Ana Pavla Almeida Diniz Gurgel^k, Henrique Douglas Melo Coutinho^{l,*}

^a Laboratory of Molecular Immunology, School of Life Sciences, Fudan University, Shanghai 200433, China

^b Department of Zoology, University of Gujrat, Gujrat, Punjab 50700, Pakistan

^c Department of Zoology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

^d Department of Zoology, Government College University, Lahore 54000, Pakistan

^e Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

^f Department of Zoology, University of Narowal, Narowal 51600, Pakistan

^g Department of Geography, Government College University, Lahore 54000, Pakistan

^h Department of Surgical Technology, Ferdows School of Allied Medicine and Public Health, Birjand University of Medical Sciences, Birjand 97178, Iran

ⁱ Ferdows School of Paramedical and Health, Birjand University of Medical Sciences, Birjand 97178, Iran

^j CECAPE College, Juazeiro do Norte 63024-015, Brazil

^k Department of Engineering and Environment, Federal University of Paraiba, João Pessoa 58051-900, Brazil

^l Laboratory of Microbiology and Molecular Biology (LMBM), Department of Biological Chemistry, Regional University of Cariri, Crato 63105-000, Brazil

ARTICLE INFO

Keywords:

Genotypes

HCV

Molecular evolution

Pakistan

Spatial distribution

ABSTRACT

Background: Hepatitis C, caused by the Hepatitis C Virus (HCV), is the second most common form of viral hepatitis. The geographical distribution of HCV genotypes can be quite complex, making it challenging to ascertain the most prevalent genotype in a specific area.

Methods: To address this, a review was conducted to determine the prevalence of HCV genotypes across various provinces and as a whole in Pakistan. The scientific literature regarding the prevalence, distribution, genotyping, and epidemiology of HCV was gathered from published articles spanning the years 1996–2020.

Results: Genotype 1 accounted for 5.1% of the patients, with its predominant subtype being 1a at 4.38%. The frequencies of its other subtypes, 1b and 1c, were observed to be 1.0% and 0.31% respectively. Genotype 2 had a frequency of 2.66%, with the most widely distributed subtype being 2a at 2.11% of the patients. Its other subtypes, 2b and 2c, had frequencies of 0.17% and 0.36% respectively. The most prevalent genotype among all isolates was 3 (65.35%), with the most frequent subtype being 3a (55.15%), followed by 3b (7.18%). The prevalence of genotypes 4, 5, and 6 were scarce in Pakistan, with frequencies of 0.97%, 0.08%, and 0.32% respectively. The prevalence of untypeable and mixed genotypes was 21.34% and 3.53% respectively. Estimating genotypes proves to be a productive method in assisting with the duration and selection of antiviral treatment. Different HCV genotypes can exhibit variations in their response to specific antiviral treatments. Different genotypes may have distinct natural histories, including variations in disease progression and severity. Some genotypes may lead to more rapid liver damage, while others progress more slowly.

Conclusions: This information can guide screening and testing strategies, helping to identify individuals at higher risk of developing severe complications. Studying the distribution of HCV genotypes in a population can provide valuable insights into the transmission dynamics of the virus.

* Corresponding authors.

E-mail addresses: 20110700169@fudan.edu.cn (A. Habib), hdmcoutinho@gmail.com (H.D.M. Coutinho).

<https://doi.org/10.1016/j.imj.2023.11.007>

Received 9 September 2023; Received in revised form 9 November 2023; Accepted 15 November 2023

2772-431X/© 2023 The Author(s). Published by Elsevier Ltd on behalf of Tsinghua University Press. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Globally hepatitis C virus is the most infected health dilemma. Hepatitis C virus belongs to the family Flaviviridae and the genus Hepacivirus [1]. HCV is considered the second most affected viral hepatitis and it is a worldwide public health issue [2]. Hepatitis C virus has been considered hepatotropic as well as a lymphotropic viral disease [3]. Hepatitis C is the inflammation of the liver which is caused by the Hepatitis C virus (HCV). It is responsible for acute as well as chronic liver infection which might range from mild type infection which remains for a few weeks and then become serious that is lethal to life persistency. Liver cancer is also caused by the infection of HCV. Most commonly individuals become infected with HCV by exposure to a small volume of blood because it is a blood-borne virus. The infection may be transmitted through unhygienic injection, unhygienic health care system, transfusion of infected blood and blood products, tattooing, skin piercing, and sexual contact which manifests in the blood. It is estimated that 71 million individuals get infected with HCV infection worldwide. Statistically, several chronically infected individuals lead to liver cirrhosis or carcinoma [4]. In 2019, the World Health Organization (WHO) reported that HCV caused around 290,000 deaths, primarily from cirrhosis and hepatocellular cancer (HCC). Globally it is also a major health issue regarding HCV infection that causes liver cirrhosis and hepatocellular carcinoma which ultimately leads to morbidity and mortality [5]. It is estimated that 350,000 mortalities occur due to the pathological complications of hepatocellular carcinoma caused by HCV worldwide [6]. It can be correlated that the high mortality rate of HCV-infected people is due to continuous and untreated HCV manifestation which leads to the progression of HCC [7]. The major risk factor for the progression of hepatocellular carcinoma is the infection of HCV and genotype 1b is the most familiar to HCC-infected individuals [8]. The occurrence of HCV chronic infection which leads to the evolution of HCC also depends on different host and atmospheric agents that include viral load, virus modification, alcohol intake, age and gender [9].

HCV prevalence in developing countries like Pakistan has continually increased significantly with the different risk factors like lack of awareness, unhygienic health care system, unsafe blood transfusions, tattooing, skin piercing, unhygienic surgical instruments, and lack of medical facilities [10]. HCV prevalence in highly affected groups is 66% which is much greater compared to the general group (3.13%–23.83%) and blood donors (1.05%–20.8%) [11]. HCV is a silent infection due to early asymptomatic infection and the infected individuals remain unsuspecting but later abnormal changes in the liver enzymes cause hepatic injury which leads to the symptomatic infection. Hepatitis C virus is most prevalent in underprivileged

countries as compared to developed countries due to different risk factors related to health care systems. However, clinical research trials are ongoing on the production of an effective vaccine for the treatment of HCV infection.

2. Molecular evolution of HCV

Hepatitis C virus is an enveloped RNA virus that was first time identified by Choo et al. in 1989 [12]. But before this, HCV was called non-A, non-B hepatitis and belonged to the family Flaviviridae which also involves the Dengue virus [12,13]. However, finally, the Hepacivirus family for HCV was developed which has low sequence homology in contrast to other flaviviruses [14]. The Hepacivirus family also includes some different hepatotropic viruses like A, B, D, E, and G [15]. HCV genetic sequence is a highly fluctuating genome consisting of many different genotypes with 9.6 Kb lengths and was first identified in 1989 [16]. These different kinds of genotypes have increased the rate of mutation as modification in the alignment that is 1.44×10^{-3} nucleotide per position annually. Meanwhile, it has been characterized as an evolutionary rate of 7.4×10^{-4} nucleotide modification per area annually for the E1 gene and 4.1×10^{-4} for the NS5B gene [17]. The genome of HCV contained a dimensional single-stranded RNA molecule of positive charge. A huge polyprotein is encoded by RNA which is approximately 3000 amino acid sequences in a single constant open reading frame (ORF) that is flanked at the 5' and 3' ends with nontranslated regions (5' UTR) [18]. This open reading frame contained 3 structural genes C, E1, E2, and 4 nonstructural genes NS2, NS3, NS4, and NS5 [19]. The genome of HCV has different mutations with different positions. Two positions of HCV envelope E2 glycoprotein have an increased rate of mutation which are abbreviated with hypervariable positions 1 and 2. It has been identified that 5' UTR in the HCV genome is the highly conserved region which is why most laboratories use this for the manufacturing of sensitive diagnostic testing HCV RNA kits [20].

One more study observed that the RNA genome showed significant heterogenetic variations with nucleotide interchange values of 1.44×10^{-3} and 1.92×10^{-3} per position annually [21]. Genetic variability of HCV isolates consists of 4 levels the first one is types with 65.7%–8.9% nucleotide sequence, the second one is subtypes with 76.9%–80.1% nucleotide sequence, the third and fourth is isolates and quasispecies with 90.8%–99% nucleotide sequence. Clinically and epidemiologically HCV genome is categorized into 11 genotypes and more than 100 subtypes assigned as 1a, 1b, 1c. etc. [22]. However, it has been observed that genotypes 7–11 of different strains are considered in the same group and categorized into a single genotype 6 [23]. Globally it is a

Table 1
Province wise percentage of HCV prevalence in Pakistan.

Province	Frequency	Percentage
Punjab	14,825	34.13
Sindh	4339	9.99
Khyber Pakhtunkhwa	3382	7.78
Balochistan	968	2.22

major challenge for researchers and scientists to develop an effective vaccine due to several HCV genotypes heterogeneity. But it is still a mystery how and when the hepatitis C virus was transmitted from Ape species to the human community. Moreover, HCV molecular and cellular phenomena with host and viral factors are not completely explored. Present identification and genotyping of HCV is being done by using molecular cloning techniques of the infected person's blood serum sample [24].

3. Literature search and maps description

Scientific literature about the prevalence, distribution, genotyping, and epidemiology of HCV was collected from different sources of published articles from 1996 to 2020. Published articles indexed in Scopus, Google Scholar, PubMed, Elsevier Wiley, etc. Articles were searched by the following keywords Prevalence, epidemiology, distribution, HCV, genotypes, molecular evolution, Pakistan, and WHO. A total of 48 articles related to the prevalence of HCV and genotypes in Pakistan were collected which covered all the provinces of Pakistan. Overall, 43,431 infected individuals with HCV were described in these articles. After the statistical analysis, 14,825 infected individuals were reported in the Punjab province which is followed by Sindh with 4339, Khyber Pakhtunkhwa reported 3382 Balochistan with 968, and 19,917 infected individuals were reported from randomly different areas of Pakistan (Table 1). The spatial distribution of HCV genotypes was determined by Interpolation IDW analysis. Interpolation is one of the basic approaches to measuring epidemiological analysis. The inverse distance weighted (IDW) method was used to measure the prevalence of HCV genotypes in Pakistan by using Arc Map 10.5. The zonal statistical method was applied after IDW interpolation and designed spatial distribution maps within the range. This geospatial technique is very helpful in understanding the spatial distribution of HCV genotypes in all the provinces of Pakistan.

4. Prevalence of HCV and its genotypes in Pakistan

The prevalence of HCV infection is endemic in Pakistan; according to a recent study, the burden of the epidemic has increased by almost 4.7%–6.8% [25]. Different factors related to the increased prevalence of HCV in Pakistan include a lack of awareness status, insufficient

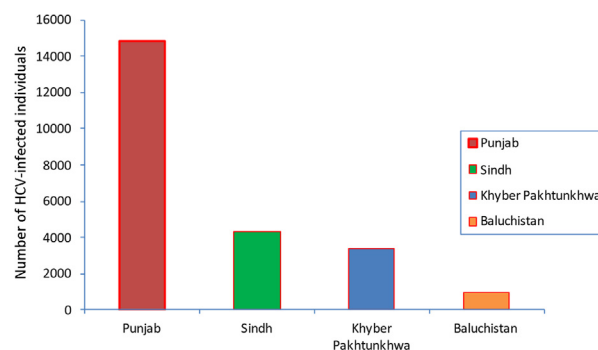


Fig. 1. Province-wise prevalence of HCV in Pakistan. KP: Khyber Pakhtunkhwa province. Statistical analysis was conducted to determine the prevalence of HCV-infected individuals across all provinces of Pakistan. Punjab province exhibited the highest prevalence at 34.13%, followed by Sindh province at 9.99%. The prevalence in Khyber Pakhtunkhwa province was recorded at 7.78%, while Balochistan showed a prevalence of 2.22%.

medical facilities, and the lack of significant data. Different studies explained the high infection rate in the different geographical areas and racial communities in Pakistan [26]. In the current review, we study more than 30 articles on the prevalence of HCV which are published in the different indexed journals. One of the major issues is the lack of testing facilities. That's why many individuals are not screened for the anti-HCV antibodies test. Furthermore, those individuals who are diagnosed with the infection do not receive medications throughout their life. The major challenge is the screening of HCV in remote areas where there is no facility for 60% population which is inhabitant [27]. The results were designed according to the data collected from different research articles. Statistical analysis was performed to measure the percentage of HCV-infected individuals in all the provinces of Pakistan. In the current review after statistical analysis, Punjab province recorded the highest prevalence (34.13%), followed by the Sindh province with 9.99%. The Prevalence in Khyber Pakhtunkhwa province (7.78%), and Balochistan 2.22% was recorded, as shown in (Figs. 1 and 2). The current review observed that limited studies have been done in remote areas and also in Balochistan which is the largest Province of Pakistan. There is a high-level demand for the screening of the anti-HCV test to measure the prevalence in these areas.

Globally HCV is the major viral disease of the liver which is endemic in the community but there is no facility for data collection related to genotypes assessment of HCV infection at a national level. Geographic distribution data of HCV genotypes is the major issue in finding out the most prevalent genotype in a particular area. This study encompasses several research efforts that focused on the geographical distribution of HCV genotypes in all the provinces of Pakistan. Pakistan shares its borders with Afghanistan and Iran to the west, India to the east, and the Arabian Sea to the south. Given this geographical context, a substantial number of individuals

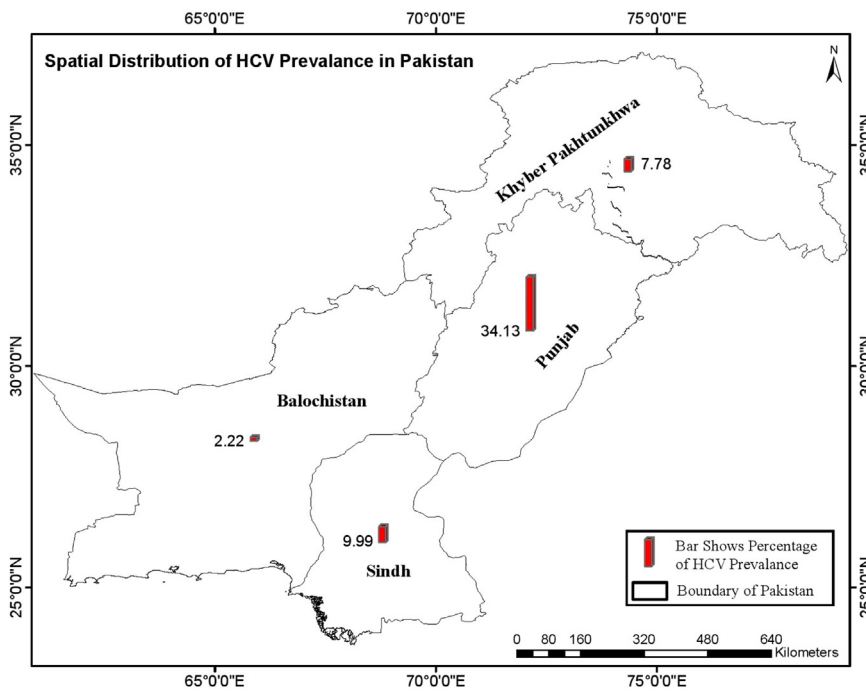


Fig. 2. Spatial distribution map of HCV prevalence in Pakistan. The prevalence of HCV genotypes in Pakistan was assessed using the Inverse Distance Weighted (IDW) method in ArcMap 10.5.

cross the border, and there is a history of unauthorized drug delivery from different territories. Previously, it has been observed that lack of precautionary measures, such as the use of unhygienic syringes, inadequate health infrastructure, unsafe blood transfusions, utilization of contaminated surgical instruments, and a lack of awareness, are all significant risk factors contributing to the prevalence of HCV in Pakistan. Pakistan has 4 provinces geographically the largest province is Balochistan with the smallest population; the second one is Punjab with the largest population, the third is Sindh and the fourth one is Khyber Pakhtunkhwa is the smallest province which shares a border with Afghanistan in the west. The countries that are highly infected with HCV are significantly involved in the modification of prevalence data by the immigration of individuals, especially in the high immigrant areas.

For a more effective analysis and treatment, detailed knowledge about the genotypes of HCV is significant, particularly about their geographical location. Many studies have revealed a correlation between genotypes and their geographical distribution, which helps measure the severity of the epidemic [28]. However, sequencing HCV genotypes is a costly procedure, which poses a challenge for assessing genotype distribution in remote areas [29]. In the past, analyses of HCV genotypes have been conducted in Pakistan, but few studies have focused on Balochistan, which is the largest province in Pakistan. In the current study, a total of 43,431 samples were studied from different published articles which showed the heterogeneous figure of genotype distribution in different areas of Pakistan (Table 2). Genotype 1 accounted for 5.1% of the patients and predominated genotype 1a was 4.38%.

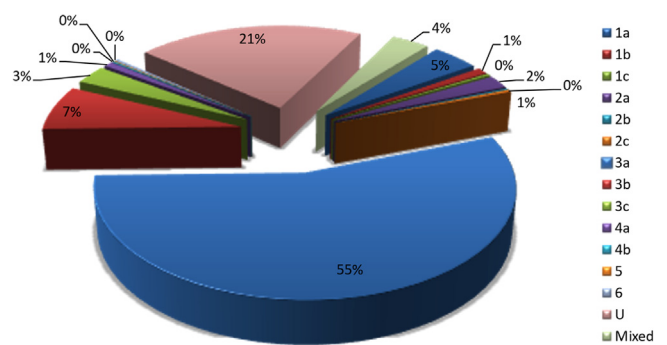


Fig. 3. Frequency distribution of HCV genotypes (subtypes) in Pakistan. Microsoft Excel was employed for the analysis of genotype prevalence in Pakistan. Among all the isolates, genotype 3 was the most prevalent, accounting for 65.35%. Within genotype 3, the most frequently observed subtype was 3a, making up 55.15% of the cases, followed by subtype 3b at 7.18%.

The frequency of genotype 1b was observed at 1.0% and 1c with 0.31% of the cases. Genotype 2 contributes the frequency of 2.66% and subtype 2a was distributed with 2.11% of the patients. Genotype 2 was observed at 0.17% and 2c was observed at 0.36%. The most prevalent genotype of all the isolates was 3 (65.35%) in the most frequent subtype was 3a (55.15) followed the 3b (7.18%) (Table 3 and Fig. 3). The prevalence of genotypes 4, 5, and 6 are rare in Pakistan with the ratio of 0.97%, 0.08%, and 0.32%, respectively (Fig. 4). The prevalence of Untypeable and mixed genotype was 21.34% and 3.53%, respectively (Fig. 5). The frequency of untypeable genotypes was observed in only 9272 infected individuals and the frequency of mixed genotypes was 1537 infected individuals. The current study revealed that HCV prevalence in hepatitis individuals is allocated predominantly

Table 2
Distribution of HCV genotypes in previous studies of Pakistan from 1996 to 2017.

Ref	HCV genotype frequency distribution															year
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	5	6	U	Mix	
[30]	1	–	–	–	–	–	13	–	–	–	–	–	–	1	–	1996
[31]	4	–	–	1	–	–	39	–	–	1	–	–	–	–	–	1997
[32]	18	–	–	3	–	–	171	–	–	–	–	2	63	–	–	2002
[33]	31	–	–	6	–	–	198	–	–	1	–	7	51	6	–	2002
[34]	20	–	–	–	–	–	–	–	–	15	–	–	–	64	–	2003
[35]	–	–	–	–	–	–	314	–	–	–	–	–	–	–	–	2002
[36]	–	–	–	–	–	–	8	1	–	–	–	–	–	–	–	2003
[37]	57	–	–	8	–	–	–	–	–	36	–	–	6	–	–	2005
[38]	–	–	–	3	–	–	62	8	–	–	–	–	–	11	–	2006
[39]	–	21	–	–	–	–	80	43	–	–	–	–	–	5	4	2008
[40]	10	–	–	–	–	–	18	–	–	–	–	–	–	–	6	2007
[41]	–	–	–	–	6	–	–	–	–	–	–	–	–	2	4	2007
[42]	–	–	80	–	–	154	–	–	1220	–	46	–	–	106	51	2007
[43]	280	101	5	252	–	3	1664	592	25	–	–	6	4	201	161	2008
[44]	10	5	–	–	–	–	242	19	–	–	–	3	–	52	9	2008
[20]	2	–	–	–	–	–	14	3	–	–	–	–	–	9	–	2009
[45]	–	10	–	–	–	–	107	33	–	–	–	–	–	8	50	2008
[46]	–	10	–	–	–	–	–	–	–	–	–	–	–	8	19	2009
[47]	4	–	–	–	–	–	115	6	–	–	–	–	–	34	41	2009
[29]	1	–	–	–	–	–	81	–	–	–	–	–	–	9	2	2009
[48]	–	–	–	–	–	–	8	–	–	–	–	–	–	11	2	2009
[49]	8	2	–	–	–	–	34	13	–	–	–	–	–	2	24	2009
[50]	2	1	2	1	–	–	105	12	3	3	–	–	–	–	–	2009
[1]	12	6	1	8	–	–	58	24	–	–	–	–	–	3	7	2010
[51]	678	170	49	431	48	3	12,537	1834	50	101	–	13	12	–	965	2010
[52]	9	3	–	–	–	–	234	25	–	–	–	–	–	116	28	2011
[53]	–	–	–	1	–	–	28	4	–	–	–	5	5	–	–	2010
[21]	322	–	–	–	–	–	763	43	–	170	16	–	–	34	16	2010
[54]	1	2	–	4	1	–	11	3	–	–	–	–	–	3	–	2010
[2]	3	5	–	78	2	–	62	16	–	–	–	–	–	34	–	2010
[55]	5	–	–	22	–	–	18	–	9	–	–	–	–	9	–	2011
[22]	10	–	–	15	–	–	63	13	–	–	–	–	–	70	14	2011
[56]	1	–	–	–	–	–	17	3	–	–	–	–	–	1	1	2011
[57]	3	–	–	–	–	–	15	4	–	–	–	–	–	1	–	2011
[58]	10	–	–	–	–	–	160	1	–	1	–	–	–	–	5	2013
[59]	6	2	–	–	–	–	57	14	–	3	–	2	–	2	–	2017
[2]	1	1	–	–	–	–	80	6	–	–	–	–	–	–	7	2010
[54]	12	11	–	12	12	–	47	11	–	–	–	–	–	11	–	2011
[60]	21	14	–	4	–	–	166	8	–	–	–	–	–	–	25	2011
[58]	10	–	–	–	–	–	160	1	–	–	1	–	–	–	5	2013
[61]	109	–	–	12	–	–	3657	–	–	5	–	–	–	–	35	2017
[62]	15	9	–	–	–	–	200	25	–	–	–	–	–	45	22	2017
[63]	11	–	–	4	–	–	45	5	–	–	–	–	–	–	–	2017
[64]	1	–	–	5	–	–	83	–	–	–	–	–	–	–	1	2020
[65]	9	9	–	40	5	–	355	14	–	–	–	–	–	101	3	2014
[66]	136	47	–	7	–	–	758	334	–	–	–	–	–	189	–	2016
[67]	26	8	–	3	2	–	714	2	–	–	–	1	–	107	24	2014
[68]	47	–	–	–	–	–	395	–	–	24	–	–	–	–	6	2014

to genotype 3 in Pakistan with a ratio of 54.56% in Punjab, 46.15% in Khyber Pakhtunkhwa 4.25%, and Sindh, 1.71% in the Balochistan province. After genotype 3 the high prevalence of untypeable genotype (21.34%) was observed which followed genotype 1(5.71%) in Pakistan (Fig. 6 and Table 4).

Statistically, no differences were observed in terms of gender and age, although many other studies have examined these factors. It is worth noting that epidemiological data from developed countries like the USA have shown a historical correlation between age and gender, primarily due to a large number of young individuals addicted to drugs [69]. According to the current epidemiological data, genotype 3 is suggested to be the dominant genotype in Pakistan, with other dominant genotypes being 1 and untypeable. It was observed that the high preva-

lence of genotype 3 allows for a better understanding of the HCV genotype frequency in Pakistan, ultimately providing valuable knowledge for the treatment and control of further HCV infections [46]. The treatment duration for genotype 3 is much shorter compared to genotype 1, and it also comes at a lower cost with fewer side effects [70]. The predominance of genotype 3 in our community confirms its prevalence in neighboring countries such as China, India, Bangladesh, and Iran [30]. In Pakistan, each genotype has several subtypes: 3 for genotype 1 (1a, 1b, 1c); 3 for genotype 2 (2a, 2b, 2c); 4 for genotype 3 (3a, 3b, 3c, 3d); 2 for genotype 4 (4a, 4b); and genotypes 5 and 6 each have 1 subtype, indicating lower heterogeneity in Pakistan. Genotype 1 is the second most prevalent genotype in Pakistan and is linked with Balochistan, which shares a border with Iran where genotypes 1 and 3 are

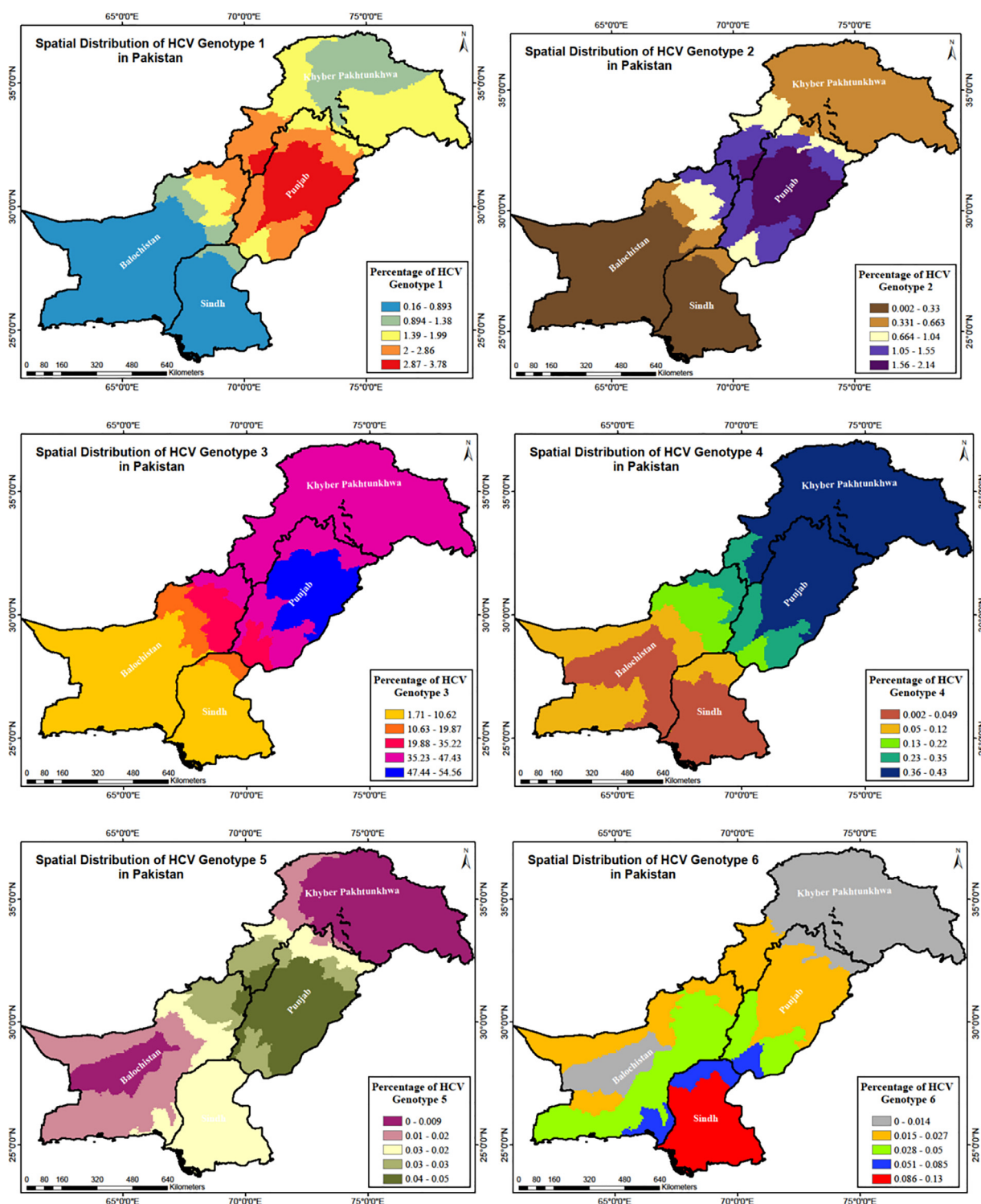


Fig. 4. Spatial distribution map of HCV genotypes/subtypes in Pakistan. The prevalence of HCV genotypes in Pakistan was assessed using the Inverse Distance Weighted (IDW) method in ArcMap 10.5. Each map represents the prevalence of HCV genotypes/subtypes.

most prevalent [31]. A high frequency of genotypes 1b and 3a has also been observed in China [32]. The distribution of genotype 1 in our country may be influenced by neighboring countries due to immigration patterns of local communities. We must take serious steps to reduce the transmission of HCV genotypes, as some, like 1a and 4a,

are potential factors contributing to liver cirrhosis. The frequency of untypeable genotypes (21.34%) suggests the presence of novel genotypes in Pakistan. Afzal et al. mentioned that a high level of mutations in the HCV genome, among other factors, may contribute to the emergence of these untypeable subtypes [33]. It is now imperative to

Table 3
Distribution of HCV genotypes and subtypes in Pakistan.

Genotype	Frequency	Percentage
1 all subtypes	2480	5.71
1a	1906	4.38
1b	437	1.0
1c	137	0.31
2 all subtypes	1156	2.66
2a	920	2.11
2b	76	0.17
2c	160	0.36
3 all subtypes	28,383	65.35
3a	23,956	55.15
3b	3120	7.18
3c	1307	3.0
4 all subtypes	423	0.97
4a	360	0.82
4b	63	0.14
5	39	0.08
6	141	0.32
Untypeable	9272	21.34
Mixed	1537	3.53
Total	43,431	100

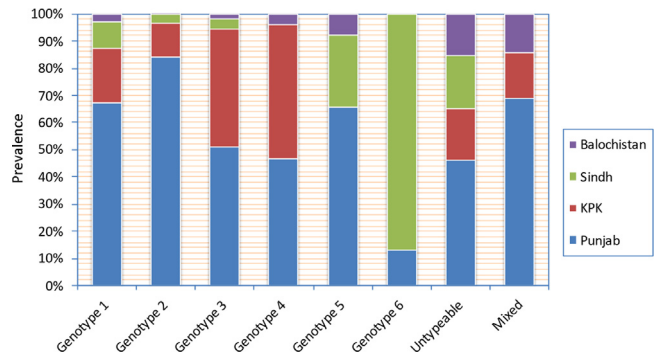


Fig. 6. Province-wise distribution of HCV genotypes in Pakistan. All the genotypes mentioned, from 1 to 6, are represented by U (untypeable), and M denotes a mixed genotype. After genotype 3 the high prevalence of untypeable genotype (21.34%) was observed which followed genotype 1 (5.71%) in Pakistan.

sequence the samples with untypeable genotypes to confirm the presence of these novel genotypes and to analyze their treatment requirements. Improving the methodology for samples that were not initially assigned specific genotypes will facilitate a more comprehensive understanding of HCV genotype prevalence and its evolutionary correlation in Pakistan.

5. Genotype distribution impact on disease management

The distribution of genotypes in a population can have important interference with the prevention and control of HCV for various reasons. Different HCV genotypes can disclose variations in their feedback to specific antiviral therapy. Some genotypes may be more resistant to certain medications, while others may respond better. Knowing the prevalent genotypes in a specific region allows health-care providers to decide the most effective treatment alternatives. Vaccines need to be effective against a broad range of genotypes to be successful in preventing infection. Knowing which genotypes are most common in a specific area prime vaccine research and development efforts. Different genotypes may have coherent natural histories, including imbalances in disease progression and severity. Some genotypes may lead to more meteoric liver damage, while others progress more slowly. Such information can help screening and testing approaches, helping to identify individuals at higher risk of developing severe complications. Studying the distribution of HCV genotypes in a population can provide a valuable understanding of the transmission dynamics of the virus. Furthermore, such information can help public health officials track and respond to outbreaks, as well as implement targeted prevention efforts. Tailoring prevention and control approaches based on genotype distribution can be more cost-effective and efficient. If a certain genotype is more prevalent in a particular population or region, resources can be directed towards targeted interventions, such as outreach and education campaigns. Some geno-

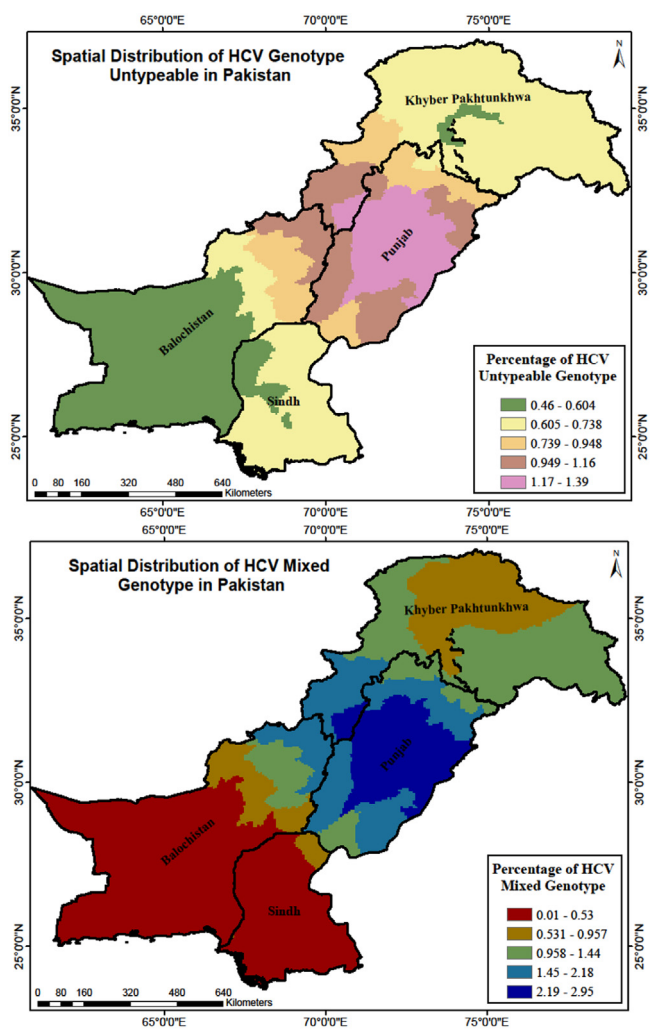


Fig. 5. Spatial distribution map of untypeable and mixed genotype of HCV in Pakistan.

Table 4
Province wise frequency of HCV genotypes in Pakistan.

Province	Province wise HCV genotypes frequency (%)							
	1	2	3	4	5	6	Untypeable	Mixed
Punjab	1	–	13	–	–	–	1	–
	4	1	39	1	–	–	–	–
	18	3	171	–	–	2	63	–
	–	–	9	–	–	–	–	–
	21	–	123	–	–	–	5	4
	–	–	–	–	–	–	2	4
	80	154	1220	46	–	–	106	51
	386	225	2281	–	6	4	201	161
	1	–	81	–	–	–	9	2
	8	–	–	–	–	–	11	2
	10	–	47	–	–	–	2	24
	897	482	14,421	101	13	12	–	965
	8	–	71	3	2	–	2	–
	109	12	3657	5	–	–	–	35
	1	5	83	–	–	–	–	1
18	45	369	–	–	–	101	3	
34	5	716	–	1	–	104	24	
47	–	395	24	–	–	–	6	
Khyber Pakhtunkhwa	10	–	140	–	–	–	8	50
	10	–	–	–	–	–	8	19
	19	8	82	–	–	–	3	7
	12	–	259	–	–	–	116	28
	322	–	806	186	–	–	34	16
	3	5	14	–	–	–	–	–
	8	80	78	–	–	–	–	34
	10	15	76	–	–	–	70	14
	1	–	20	–	–	–	1	1
	3	–	19	–	–	–	1	–
	10	–	161	1	–	–	–	5
	2	–	86	–	–	–	–	7
	23	24	58	–	–	–	11	–
	38	4	174	–	–	–	–	125
	10	–	161	1	–	–	–	5
11	4	50	–	–	–	–	–	
Sindh	18	3	171	–	–	2	63	–
	31	6	198	1	7	51	6	–
	–	–	314	–	–	–	–	–
	10	–	18	–	–	–	–	6
	–	1	32	–	5	5	–	–
	5	22	27	–	–	–	9	–
	183	7	1092	–	–	–	189	–
Baluchistan	20	–	–	15	–	–	64	–
	15	–	261	–	3	–	52	9
	2	–	17	–	–	–	9	–
	4	–	121	–	–	–	34	41
	5	1	123	–	–	–	–	–
	24	–	225	–	–	–	45	22

types may be more easily transmitted via blood transfusions or organ transplants. Adjusting screening methods based on genotype prevalence can help minimize the risk of transmission through these routes. In summary, understanding the prevalence of HCV genotypes in a population is important for tailoring prevention and control efforts to effectively combat the spread of the virus. It allows for the development of targeted interventions, optimization of treatment approaches, and the design of vaccines that can offer broad protection against various genotypes [15].

6. Conclusions

The current study presents a pooled analysis of HCV prevalence distribution in Pakistan. Genotype 3 emerges

as the most prevalent genotype, followed by untypeable strains and genotype 1. Determining genotypes is a crucial method for guiding the duration and selection of antiviral treatments. Nevertheless, there is a lack of facilities for analyzing untypeable genotypes. If this were made possible, it could lead to the identification of novel genotypes in Pakistan. However, various factors such as low awareness levels, insufficient medical facilities, and the high cost of treatment pose significant barriers to reducing HCV prevalence in Pakistan. Moreover, there is a pressing need to enhance awareness levels, bolster medical infrastructure, and implement nationwide screening programs in remote areas. Additionally, establishing comprehensive hepatitis clinical research centers is imperative.

7. Future perspective

The perspective of our work is to serve as a foundation for public health measures aimed at preventing the spread of these genotypes of HCV. We aim to avoid the proliferation of the most virulent strains, which can have a significant impact on the well-being and quality of life of the people of Pakistan.

Funding

None.

Author contributions

Conceptualization and Writing original draft: A.H., F.I.; Methodology: N.H., K.M.H., R.I., Z.H.; Formal analysis: M.H.T., M.A.; Validation: C.R.M.; Software: M.A.N., S.D.; Resources and Project administration: H.D.M.C.; Supervision: F.I. All authors read and approved the final manuscript.

Acknowledgments

None.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

Different kinds of search engines were used such as Google Scholar, PubMed, and ResearchGate to collect the relevant kinds of research and review articles for the manuscript literature.

Ethics statement

Not applicable.

Informed consent

Not applicable.

References

- Haqqi, R. Munir, M. Khalid, et al., Prevalence of hepatitis C virus genotypes in Pakistan: current scenario and review of literature, *Viral. Immunol.* 32 (9) (2019) 402–413, doi:10.1089/vim.2019.0058.
- Ali, H. Ahmed, M. Idrees, Molecular epidemiology of hepatitis C virus genotypes in khyber pakhtoonkhaw of Pakistan, *Virology* 7 (2010) 203, doi:10.1186/1743-422X-7-203.
- A.A. Akhund, K. Shaikh, S. Naqvi, HCV genotypes in correlation to histopathological grading and staging in interior Sindh, *Gomal J. Med. Sci.* 6 (2) (2008) 93–97.
- J.M. Pawlowsky, C.B. Ramers, J.F. Dillon, Simplification of care for chronic hepatitis C virus infection, *Semin. Liver Dis.* 40 (4) (2020) 392–402, doi:10.1055/s-0040-1713657.
- T. Bashir, M. Asim, M. Ahsan, A case report: patient with the history of hepatitis C virus, *J. Antivir. Antiretrovir.* 9 (2017) 1–3, doi:10.4172/1948-5964.1000162.
- A. Khaliq, I. Ahmad, M. Sohail Afzal, HCV treatment with sofosbuvir in Pakistan; current scenario and future perspective, *J. Gastroenterol. Hepatol. Res.* 7 (2) (2018) 2578–2580, doi:10.17554/j.issn.2224-3992.2018.07.770.
- A. Petruzzello, G. Loquercio, R. Sabatino, et al., Prevalence of Hepatitis C virus genotypes in nine selected European countries: a systematic review, *J. Clin. Lab. Anal.* 33 (5) (2019) e22876, doi:10.1002/jcla.22876.
- A. Petruzzello, S. Marigliano, G. Loquercio, et al., Hepatitis C Virus (HCV) genotypes distribution among hepatocellular carcinoma patients in Southern Italy: a three year retrospective study, *Infect. Agents Cancer* 12 (1) (2017) 52, doi:10.1186/s13027-017-0162-5.
- A. Petruzzello, Epidemiology of hepatitis B virus (HBV) and hepatitis C virus (HCV) related hepatocellular carcinoma, *Open Virol. J.* 12 (2018) 26–32, doi:10.2174/1874357901812010026.
- A. Habib, K.S.B. Razzak, M. Imran, et al., Elimination of Hepatitis C Virus: a goal of WHO, *Vigyan Varta* 1 (2020) 56–59.
- M. Umer, M. Iqbal, Hepatitis C virus prevalence and genotype distribution in Pakistan: comprehensive review of recent data, *World J. Gastroenterol.* 22 (4) (2016) 1684–1700, doi:10.3748/wjg.v22.i4.1684.
- Q.L. Choo, G. Kuo, A.J. Weiner, et al., Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome, *Science* 244 (4902) (1989) 359–362, doi:10.1126/science.2523562.
- S. Riaz, A. Iqbal, A review of hepatitis C in the general population in Pakistan, *Viral Hepat. Dergisi* 22 (3) (2016) 74–81, doi:10.4274/vhd.96729.
- A. Zidan, H. Scheuerlein, S. Schüle, et al., Epidemiological pattern of hepatitis B and hepatitis C as etiological agents for hepatocellular carcinoma in Iran and worldwide, *Hepat. Mon.* 12 (10 HCC) (2012) e6894, doi:10.5812/hepatmon.6894.
- A.G. Lim, J.G. Walker, N. Mafirakureva, et al., Effects and cost of different strategies to eliminate hepatitis C virus transmission in Pakistan: a modelling analysis, *Lancet Glob. Health* 8 (3) (2020) e440–e450, doi:10.1016/S2214-109X(20)30003-6.
- E. Giang, M. Dörner, J.C. Prentoe, et al., Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus, *Proc. Natl. Acad. Sci. USA* 109 (16) (2012) 6205–6210, doi:10.1073/pnas.1114927109.
- Guidelines for the screening, care and treatment of persons with hepatitis C infection, WHO, 2018, p. 108. Available from: <https://www.who.int/publications/i/item/9789241548755> (accessed on 13 November 2023).
- M.S. Alfaresi, Prevalence of hepatitis C virus (HCV) genotypes among positive UAE patients, *Mol. Biol. Rep.* 38 (4) (2011) 2719–2722, doi:10.1007/s11033-010-0415-5.
- A. Mukhopadhyaya, Hepatitis C in India, *J. Biosci.* 33 (4) (2008) 465–473, doi:10.1007/s12038-008-0065-0.
- S. Afridi, M. Naeem, A. Hussain, et al., Prevalence of hepatitis C virus (HCV) genotypes in Balochistan, *Mol. Biol. Rep.* 36 (6) (2009) 1511–1514, doi:10.1007/s11033-008-9342-0.
- N. Ullah, M.A. Kakakhel, Y. Bai, et al., Prevalence of active HCV infection and genotypic distribution among the general population of district Mardan, Pakistan, *Braz. J. Biol.* 83 (2021) e244977, doi:10.1590/1519-6984.244977.
- Z. Qamar, F. Anwar, R. Ahmad, et al., Prevalence of Hepatitis C virus and determination of its genotypes in subjects of Tehsil Daggar District Buner, KP, Pakistan, *Clin. Epidemiol. Glob. Health.* 12 (2021) 100809, doi:10.1016/j.cegh.2021.100809.
- P. Bruggmann, T. Berg, A.L.H. Øvrehus, et al., Historical epidemiology of hepatitis C virus (HCV) in selected countries, *J. Viral Hepat.* 21 (Suppl 1) (2014) 5–33, doi:10.1111/jvh.12247.
- A. Furuta, K.A. Salam, N. Akimitsu, et al., Cholesterol sulfate as a potential inhibitor of hepatitis C virus NS3 helicase, *J. Enzyme Inhib. Med. Chem.* 29 (2) (2014) 223–229, doi:10.3109/14756366.2013.766607.
- Z. Khan, M. Shafiq, F. Shahab, Frequency and risk factors of hepatitis b & c in Afghan patients presenting to tertiary care hospital in Peshawar, Pak. *Armed Forces Med. J.* 65 (5) (2015) 686–689.
- S.A. Junejo, N. Khan, A. Ali Lodhi, Prevalence of hepatitis band C infection in patients admitted at tertiary eye care centre: a hospital based study, *Pak. J. Med. Sci.* 25 (4) (2009) 597–600.
- S. Ali, A. Ahmad, R.S. Khan, Genotyping of HCV RNA reveals that 3a is the most prevalent genotype in Mardan, Pakistan, *Adv. Virol.* 2014 (2014) 606201, doi:10.1155/2014/606201.
- A. Kabir, S.M. Alavian, H. Keyvani, Distribution of hepatitis C virus genotypes in patients infected by different sources and its correlation with clinical and virological parameters: a preliminary study, *Comp. Hepatol.* 5 (2006) 4, doi:10.1186/1476-5926-5-4.
- A. Yousaf, A. Ghafoor, N. Fatima, et al., Gender-specific frequency distribution of hepatitis C virus genotypes in Punjab Province, Pakistan: a clinically significant descriptive cross-sectional study, *Cureus* 13 (8) (2021) e17480, doi:10.7759/cureus.17480.
- A. Khan, A. Nadir, M.H. Mushtaq, et al., Molecular epidemiology and genotype distribution of hepatitis C in Pakistan; a multicenter cross-sectional study, *Infect. Genet. Evol.* 84 (2020) 104372, doi:10.1016/j.meegid.2020.104372.
- U. Saleem, N. Aslam, R. Siddique, et al., Hepatitis C virus: its prevalence, risk factors and genotype distribution in Pakistan, *Eur. J. Inflamm.* 20 (2022), doi:10.1177/1721727X221144391.
- S.J. Zuberi, A. Arif, Serotyping of the hepatitis C in Pakistan, *J. Pak. Med. Assoc.* 52 (5) (2002) 218–219.

- [33] N. Ansari, A. Ahmed, J. Esmail, et al., HCV serotypes in Karachi: a liaquat national hospital experience, *J. Pak. Med. Assoc.* 52 (5) (2002) 219–220.
- [34] N. Khokhar, N. Asif, O.S. Khokhar, Serotype 3 is most common hepatitis C serotype in Pakistan: however, significant numbers are untypeable, *Hepatology* 38 (1) (2003) 270–271, doi:10.1053/jhep.2003.50260.
- [35] S. Akhtar, T. Moatter, Hepatitis C virus infection in polytransfused thalassemic children in Pakistan, *Indian Pediatr.* 41 (10) (2004) 1072–1073.
- [36] N. Hussain, S. Bungau, T. Behl, Distribution of hepatitis C virus genotypes in Punjab region, Pakistan, based on a study of 4177 specimens, *Infect. Genet. Evol.* 91 (2021) 104811, doi:10.1016/j.meegid.2021.104811.
- [37] K. Mumtaz, S.S. Hamid, T. Moatter, et al., Distribution of hepatitis C virus genotypes and its response to treatment in Pakistani patients, *Saudi Med. J.* 29 (11) (2008) 1671–1673.
- [38] M. Siddique, M. Shafique, A. Mateen, et al., Prevalence and genotypic analysis of hepatitis C virus isolated from liver center, Faisalabad, Pakistan, *Pakistan J. Zool.* 52 (2020) 313.
- [39] T. Ijaz, M.K. Shahzad, N. Sarfraz, et al., Prevalence of Genotype 3a Hepatitis C Virus (HCV) In the Infected Population of Lahore, Pakistan, *IJAVMS* 2 (2008) 14–17.
- [40] S. Anis, R. Muzaffar, E. Ahmed, Cryoglobulinaemia and autoimmune markers in hepatitis C virus infected patients on renal replacement therapy, *J. Pak. Med. Assoc.* 57 (5) (2007) 225–229.
- [41] A. Ahsan, A.Z. Khan, H. Javed, Estimation of hepatitis C prevalence in the Punjab Province of Pakistan: a retrospective study on general population, *PLoS One* 14 (4) (2019) e0214435, doi:10.1371/journal.pone.0214435.
- [42] F. Mahmood, S. Kazmi, H. Wakil, et al., Genotype base prevalence of hepatitis C, *Pak. J. Med. Health Sci* 16 (07) (2022) 664.
- [43] M. Idrees, S. Riazuddin, Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission, *BMC Infect. Dis.* 8 (2008) 69, doi:10.1186/1471-2334-8-69.
- [44] A.A. Akhund, K.R. Shaikh, S.Q.H. Naqvi, et al., HCV genotypes in correlation to histopathological grading and staging in interior Sindh, *Gomal J Med Sci* 6 (2) (2008).
- [45] S. Hakim, S. Kazmi, O. Bagasra, Seroprevalence of hepatitis B and C genotypes among young apparently healthy females of Karachi-Pakistan, *Libyan J. Med.* 3 (2) (2008) 66–70, doi:10.4176/071123.
- [46] A. Ahmad, B. Ahmad, A. Ali, et al., Seroprevalence of HBsAg and anti-HCV in general healthy population of swat district with frequency of different HCV genotypes, *Pak. J. Med. Sci.* 25 (2009) 744–748.
- [47] S.Z. Abbas, M. Ali, A.H. Muhammad, et al., Frequency of HCV infection and its genotypes among patients attending a liver clinic and voluntary blood donors in a rural area of Pakistan, *Pak J Med Sci* 25 (2009) 579–582.
- [48] N. Asif, T. Zafar, K. Hassan, et al., Seroprevalence Anti HCV antibodies, HCV-RNA and its genotypes among patients of hemophilia, at hemophilia treatment centre Pakistan Institute of Medical Sciences, Islamabad, *Int J Pathol* (2009) 84–87.
- [49] M. Idrees, S. Rafique, I. Rehman, et al., Hepatitis C virus genotype 3a infection and hepatocellular carcinoma: Pakistan experience, *World J. Gastroenterol.* 15 (40) (2009) 5080–5085, doi:10.3748/wjg.15.5080.
- [50] A. Khan, Y. Tanaka, Z. Azam, et al., Epidemic spread of hepatitis C virus genotype 3a and relation to high incidence of hepatocellular carcinoma in Pakistan, *J. Med. Virol.* 81 (7) (2009) 1189–1197, doi:10.1002/jmv.21466.
- [51] S. Butt, M. Idrees, H. Akbar, et al., The changing epidemiology pattern and frequency distribution of hepatitis C virus in Pakistan, *Infect. Genet. Evol.* 10 (5) (2010) 595–600, doi:10.1016/j.meegid.2010.04.012.
- [52] K. Munir, M.A. Khan, A.M. Aftab, et al., Epidemiological and Genotypical Features of HCV in District Upper Dir, Khyber Pakhtunkhwa, Pakistan, *J. Microbiol. Infect. Dis* 11 (04) (2021) 217–223.
- [53] S.T. Hakim, S. Noorali, M. Ashby, et al., Seroprevalence of co-infection of hepatitis B and hepatitis C genotypes among adult female population of Karachi, Pakistan, *Br J Med Pract* 3 (3) (2010) a335.
- [54] M. Numan, M. Jabbar, A. Zafar, et al., Prevalence, genotypic distribution and the associated risk factors of hepatitis c infection in Pakistan pediatric patients, *J. Pure Appl. Microbiol* 16 (1) (2022) 130–137.
- [55] I.U. Rehman, M. Idrees, M. Ali, Hepatitis C virus genotype 3a with phylogenetically distinct origin is circulating in Pakistan, *Genet. Vaccines Ther.* 9 (1) (2011) 2, doi:10.1186/1479-0556-9-2.
- [56] S. Khan, S. Attaullah, S. Ayaz, et al., Molecular epidemiology of HCV among health care workers of Khyber Pakhtunkhwa, *Virol. J.* 8 (8) (2011) 105, doi:10.1186/1743-422X-8-105.
- [57] M. Idrees, A. Lal, F.A. Malik, et al., Occult hepatitis C virus infection and associated predictive factors: the Pakistan experience, *Infect. Genet. Evol.* 11 (2) (2011) 442–445, doi:10.1016/j.meegid.2010.12.004.
- [58] S.Q. Afridi, M.N. Zahid, M.Z. Shabbir, et al., Prevalence of HCV genotypes in district Mardan, *Virol. J.* 10 (2013) 90, doi:10.1186/1743-422X-10-90.
- [59] S. Ullah, M. Idrees, S. Ullah, et al., Novel distribution of HCV genotypes and their main routs of infection in the punjab Pakistan, *Biomed. J. Sci. Tech. Res.* 1 (4) (2017) 953–955.
- [60] A. Ali, M. Nisar, H. Ahmad, et al., Determination of HCV genotypes and viral loads in chronic HCV infected patients of Hazara Pakistan, *Virol. J.* 8 (2011) 466, doi:10.1186/1743-422X-8-466.
- [61] A. Afzal, Prevalence of hepatitis C virus (HCV) genotypes in Rawalpindi and Islamabad region, *J. Rawalpindi Med. Coll.* 21 (2017) 173–175.
- [62] N. Nazir, M.R. Jan, A. Ali, et al., Prevalence of hepatitis-C virus genotypes and potential transmission risks in Malakand Khyber Pakhtunkhwa, Pakistan, *Virol. J.* 14 (1) (2017) 160, doi:10.1186/s12985-017-0829-y.
- [63] T. Kumar, N. Ahmad, M.K. Hayat, et al., Prevalence and genotypic distribution of hepatitis C Virus in Peshawar KPK, Pakistan, *Hayati J. Biosci.* 24 (2017) 22–25, doi:10.1016/j.hjb.2017.04.002.
- [64] M.U. Khan, H. Sadia, A. Irshad, et al., Detection, quantification and genotype distribution of HCV patients in Lahore, Pakistan by real-time PCR, *Afr. Health Sci.* 20 (3) (2020) 1143–1152, doi:10.4314/ahs.v20i3.16.
- [65] T.M. Khan, M.T. Mehr, H. Ullah, et al., Frequency of hepatitis C virus genotypes in the north of Pakistan, *Gomal J. Med. Sci.* 12 (2014).
- [66] S. Riaz, M.F. Bashir, S. Haider, et al., Association of genotypes with viral load and biochemical markers in HCV-infected Sindhi patients, *Braz. J. Microbiol.* 47 (4) (2016) 980–986, doi:10.1016/j.bjm.2016.07.014.
- [67] S.Q. Afridi, M.M. Ali, F. Awan, et al., Molecular epidemiology and viral load of HCV in different regions of Punjab, Pakistan, *Virol. J.* 11 (2014) 24, doi:10.1186/1743-422X-11-24.
- [68] A. Rasheed, S. Ullah, S. Naeem, et al., Occurrence of HCV genotypes in different age groups of patients from Lahore, Pakistan, *Adv. Life Sci.* 1 (3) (2014) 89–95.
- [69] S.H. Zarkesh-Esfahani, M.T. Kardi, M. Edalati, Hepatitis C virus genotype frequency in Isfahan Province of Iran: a descriptive cross-sectional study, *Virol. J.* 7 (2010) 69, doi:10.1186/1743-422X-7-69.
- [70] M.S. Afzal, M.Y. Khan, M. Ammar, et al., Diagnostically untypable hepatitis C virus variants: it is time to resolve the problem, *World J. Gastroenterol.* 20 (46) (2014) 17690–17692, doi:10.3748/wjg.v20.i46.17690.