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REVIEW

Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data

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Abstract

Hepatitis C virus (HCV) is endemic in Pakistan and its burden is expected to increase in coming decades owing mainly to widespread use of unsafe medical procedures. The prevalence of HCV in Pakistan has previously been reviewed. However, the literature search conducted here revealed that at least 86 relevant studies have been produced since the publication of these systematic reviews. A revised updated analysis was therefore needed in order to integrate the fresh data. A systematic review of data published between 2010 and 2015 showed that HCV seroprevalence among the general adult Pakistani population is 6.8%, while active HCV infection was found in approximately 6% of the population. Studies included in this review have also shown extremely high HCV prevalence in rural and underdeveloped peri-urban areas (up to 25%), highlighting the need for an increased focus on this previously neglected socioeconomic stratum of the population. While a 2.45% seroprevalence among blood donors demands immediate measures to curtail the risk of transfusion transmitted HCV, a very high prevalence in patients attending hospitals with various non-liver disease related complaints (up to 30%) suggests a rise in the incidence of nosocomial HCV spread. HCV genotype 3a continues to be the most prevalent subtype infecting people in Pakistan (61.3%). However, recent years have witnessed an increase in the frequency of subtype 2a in certain geographical sub-regions within Pakistan. In Khyber Pakhtunkhwa and Sindh provinces, 2a was the second most prevalent genotype (17.3% and 11.3% respectively). While the changing frequency distribution of various genotypes demands an increased emphasis on research for novel therapeutic regimens, evidence of high nosocomial transmission calls for immediate measures aimed at ensuring safe medical practices.

Key words: Hepatitis C; Pakistan; Hepatitis C virus; Liver cancer; Hepatitis C virus genotypes; Epidemiology

Core tip: Hepatitis C virus (HCV) in Pakistan is highly endemic, with around 6.8% of general population infected with this virus. Approximately 6% of the population of Pakistan is actively infected with HCV. However, only very few relevant reports are available and more studies are needed. Research articles reviewed suggest a link between underdevelopment and HCV prevalence, as well as the predominant involvement of unsafe medical procedures in the spread of the virus in Pakistan. Although genotype 3a is most prevalent HCV subtype in Pakistan, recent years have witnessed an increase in the incidence of genotype 2a in Sindh and Khyber Pakhtunkhwa provinces.

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INTRODUCTION

Infection with hepatitis C virus (HCV) is a major global health concern. With an estimated 170 million people infected with HCV worldwide, this disease is proving to be an escalating economic, social and health burden[1,2]. Although the prevalence of HCV infection seems to have declined in the past two decades in the United States[3,4], Western and Northern Europe[5,6], Japan[7] and Australia[8], the burden of this disease in many of the lesser developed and developing countries is continuously on the rise[2]. Awareness, improved safety of blood products, the availability of affordable and effective HCV therapies have contributed significantly to the decline in HCV in developed countries. However, lack of awareness, inadequate blood screening facilities, nosocomial transmission and a lack of effective treatments (due to various reasons) have so far been the major factors responsible for seemingly inexorable rise in HCV infection in many developing countries[2,9].

HCV is a member of the family *Flaviviridae* with an approximately 9.6 kb single-stranded, positive sense RNA genome[10]. Owing to the poor fidelity of HCV RNA-dependent-RNA-polymerase (NS5B protein), the virus exhibits a high level of sequence heterogeneity[10]. Based on sequence homology six major HCV genotypes (1-6) and numerous distinct subtypes (denoted by a small English alphabet suffixed after genotype, *e.g.*, 1b, 3a *etc.*) have so far been identified[11]. The distribution of HCV genotypes is highly variable. Genotypes 1-3 are distributed globally, whereas genotypes 4 and 5 are restricted to the Middle East and Africa, and genotype 6 occurs predominantly in south-east Asian countries[2,12-14]. HCV genotype 3 is endemic on the Indian subcontinent[1]. Multiple studies have identified subtype 3a as the most prevalent HCV variant in Pakistan[15,16]. However, recent data suggest that, although genotype 3a might still be the predominant HCV subtype in Pakistan, the epidemiological pattern and relative frequency distribution of various genotypes have undergone appreciable change[17,18]. These studies have indicated a rise in the incidence of genotype 2a, particularly in the north-western province Khyber Pakhtunkhwa (KPK)[17,18].

The prevalence of HCV varies by region. Western Europe, the Americas and Australia are considered regions of low HCV prevalence (< 2%). African and the eastern Mediterranean are areas with the highest HCV prevalence[9]. In Egypt, the prevalence of HCV is greater than 14%, the highest of any country in world[19,20]. Even though the prevalence in Asia has been estimated to be a little above 2%, it varies greatly between individual countries[1]. Mongolia has the highest HCV prevalence (above 10%), followed by Uzbekistan and Pakistan where, according to some reports, around 6% of the total population is infected with HCV[1].

Our understanding of HCV epidemiology in Pakistan has been greatly improved by the numerous studies conducted over the span of the past two decades. Additionally, a comprehensive nation-wide survey of HBV/HCV prevalence, probably the first of its kind, was carried out in the years 2007-2008[21] and a national hepatitis sentinel site surveillance system has been fully operational since June 2010[22]. Five single facility-based sentinel sites (located in four provincial capitals and Islamabad) have so far been established. Nonetheless, due to limited catchment areas of these facilities, data related to the incidence of new cases and ongoing transmission patterns and trends in the majority of the population remains inadequate. Also, the scope of the national survey was limited only to screening for HBV/HCV seropositivity among healthy individuals. Thus the survey did not encompass HCV prevalence in high risk groups and the frequency distribution of HCV genotypes was also not studied. Even a cumbersome endeavor like national survey therefore did not provide data of such high translational importance.

Data on the prevalence of HCV in Pakistan has previously been comprehensively reviewed[16,23,24]. Recent years have seen an increased focus among Pakistani researchers on the study of HCV prevalence patterns and frequency distribution of its genotypes. At least eighty six relevant studies have been published in national and international journals since the publication of aforementioned reviews (2009, 2010). These newer studies have not only explored HCV prevalence in previously uncovered areas (such as Azad Kashmir[25] and Balochistan[26,27]) but have also shed light on the possible connection between underdevelopment and high HCV prevalence (for example, 23.83% prevalence in peri-urban areas of country's largest city Karachi[28] and 25.1% in rural Sindh[29]). This highlights the importance and need for integration of newer reports

in a comprehensive updated analysis. Above all, given the fact that some 15%-45% HCV infected patients may spontaneously clear the virus but still remain seropositive for HCV[30,31], results from seroprevalence studies may end up exaggerating the actual burden of disease. Recent World Health Organization (WHO) guidelines also recommend that polymerase chain reaction (PCR) based HCV diagnosis should be carried out not only to confirm HCV seropositivity but also to distinguish persons with active HCV infection from those with resolved past infection[32]. Not much attention has been paid to the categorical estimation of active HCV infection as determined by HCV nucleic acid testing (NAT) in previous systematic reviews[16,23,24].

The primary objective of this review is to summarize recent evidence and attempt to address the deficiencies in our knowledge of the epidemiology of HCV in Pakistan. Moreover, a thorough literature search, spanning the last two decades (1994-2015), was carried out to identify studies that investigated HCV prevalence in the general adult population of Pakistan. Results from these studies were pooled to produce a comprehensive map of HCV prevalence in Pakistan at the district level (Figure 1).

METHODOLOGY

Literature search

A systematic literature search was carried out for publications dealing with the prevalence of HCV infection in the general population, blood donors, various population sub-groups considered to be at increased risk of contracting HCV infection and patients suffering from liver disease *etc*. Moreover scientific reports of the frequency distribution of HCV genotypes in Pakistan were also considered. Articles and abstracts published between 2010 and April 2015, in both indexed and non-indexed journals, were included. The literature search used the electronic databases PubMed, Google Scholar as well as PakMediNet (for non-indexed Pakistani journals). Relevant literature and unpublished data was also obtained from the Pakistan Medical Research Council website (http://www.pmrc.org.pk). All the relevant research articles that appeared after the publication of earlier compilations of HCV related Pakistani data (December 2009[16], Summer 2010[23] and December 2010[24]) were included. Any relevant papers published prior this, which were not covered in any of the previous reviews, were also included. Furthermore, references cited within articles were also carefully screened to look for more relevant publications. In total, eighty seven relevant publications were identified. Out of these, one study was excluded due to possible data duplication and eighty-six (86) studies were finally included in the review.

A few studies used two step confirmatory testing for estimating the seroprevalence of HCV; verification of the initial immunochromatographic screening (ICT) by more sensitive methods such as enzyme linked immunosorbent assay (ELISA). In such cases, only those samples were included in calculations which tested positive for both of the assays. In addition to the estimation of HCV seroprevalence, as defined by detection of antibodies against HCV in an individual's blood, studies which investigated active HCV infection in various population groups were included. Active HCV infection was defined as the presence of HCV RNA in a patient's serum, as assessed by established PCR based methods[33]. For studies that reported both seroprevalence and active HCV infection, respective results were included in both estimates. Similarly, data from studies reporting HCV prevalence (sero- or active) in more than one population group was included in both calculations.

Analysis

Results from all the studies pertaining to a specific population subset were pooled and the results are presented as the weighted average. The weighted mean was calculated using the formula previously described[16].

$$\bar{x} = \frac{\sum_{i=1}^{n} \omega_i x_i}{\sum_{i=1}^{n} \omega_i}$$

Where ω represents the number of subjects included in an individual study and x stands for percent prevalence found in that particular study.

HCV PREVALENCE IN VARIOUS POPULATION GROUPS

Seroprevalence of HCV in the general population

Data obtained from sixteen studies[26,28,29,34-46] and the national survey on the prevalence of HCV infection in Pakistan (described as the "national survey" from here on)[21] is presented in this section (Table 1). A total of 165846 persons were screened in these studies. The prevalence of HCV infection in general adult population (15 years of age and above) ranged from 2.3% in Gujranwala[43] to 28.6% in rural areas of Nausheroferoz district, Sindh[29]. Mean serofrequency of HCV infection among healthy adults in Pakistan was found to be 6.8%, significantly higher than previous estimates; 4.7%[23] and 4.95%[16] as well as 4.87% for the national survey[21]. Unfortunately no reports were available from Federally Administered Tribal Areas, Gilgit Baltistan and some districts in Balochistan and KPK. Nonetheless, studies cited in this article mainly pertained to three of the bigger provinces, Punjab, Sindh and KPK (most districts), as well as one study from Balochistan[26], which together make up for more than 80% of the population of Pakistan.

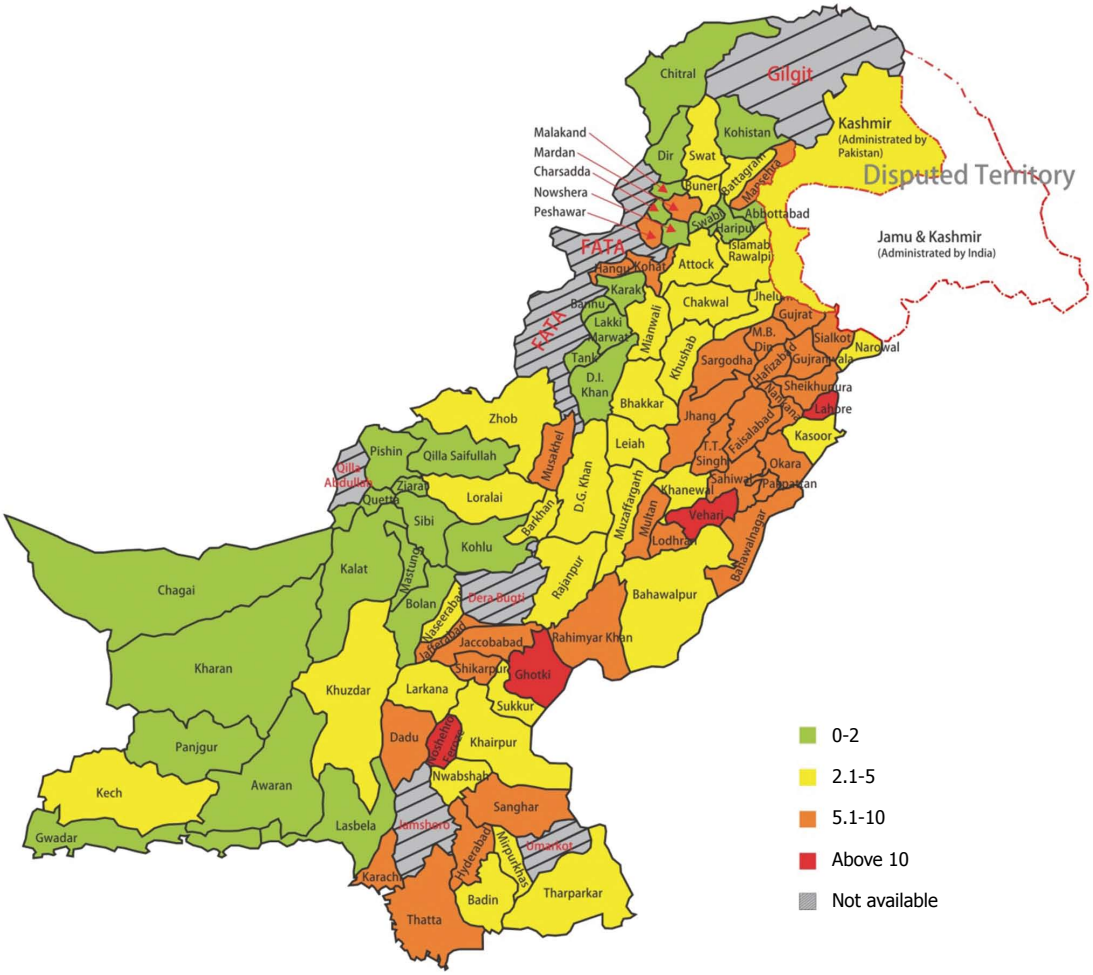

Figure 1 Hepatitis C virus seroprevalence in Pakistan at the district level.

An almost 40% increase in HCV seroprevalence shown in our analysis, in comparison to earlier reports, is alarming. However, it is not clear whether this upward trend is due to the coverage of more high risk groups, better sampling strategies and improved diagnostic facilities or actually indicates an increase in the incidence of new HCV cases. It must be noted here that in the last seven years, Pakistan has suffered at least one major natural disaster (2010 flood) as well as fallout from the ongoing war on terror in the form of mass population displacements. Effects of such human tragedies on the spread of HCV infection in an already high prevalence country cannot be overlooked and needs to be monitored carefully. At least one report can be cited in this context. A study conducted in 2010 revealed that HCV serofrequency in internally displaced persons from Swat district was indeed much higher than reported previously for the healthy adult population of the same district (8.8% *vs* 2.2%)[45,47].

It is worthwhile mentioning that subjects of most of the studies cited in the current review were either in urban areas or in some cases not clearly defined (Table 1). Nonetheless, at least two of the studies that categorically targeted rural[29] or peri-urban populations[28] reported an extremely high frequency of anti-HCV prevalence in these areas, 28.6% and 23.8% respectively (entries 5 and 6 in Table 1). In addition to this, an older study found a remarkably high HCV prevalence in the rural population of Punjab province in comparison to urbanized areas (15.73% *vs* 9.95%, respectively)[48]. Earlier reports published by Abbas *et al*[25,34] also substantiate the suspicion that the burden of HCV disease among rural areas is manifold higher than in urban areas. Unfortunately, due to scarcity of studies specifically targeting the rural population, neither the true extent of HCV spread in rural areas is known, nor does it reflect in national prevalence estimates. As more than 60% of people in Pakistan reside in these high prevalence rural areas[49], the actual burden of HCV disease in Pakistan may be much higher than current and previous estimates. More studies are needed to fill this fundamental gap in our knowledge regarding the true burden of HCV disease in Pakistan.

Previous studies as well as the national survey have indicated a highly heterogeneous pattern of HCV prevalence in various cities and regions of Pakistan. In order to obtain a more comprehensive picture of HCV prevalence in various districts and regions of Pakistan, we carried out a thorough literature search spanning

Table 1 Hepatitis C virus seroprevalence among healthy adult population

S. #	Year	Place	Method	Sample size			Seroprevalence (%)			Ref.
				Male	Female	Total	Male	Female	Total	
1	2009	Lahore	ELISA[9]	-	-	2000	-	-	6.5	[38]
2	2009	Larkana	ICT[1]	353	97	450	7.37	4.12	6.66	[46]
3	2010	Swat	ICT[2]	290	300	590	6.2	11.3	8.81	[45]
4	2010	Mansehra	ICT[2]	-	-	400	-	-	7.0	[36]
5	2010	Thatta/Nausheroferoz	ICT[3]	-	-	303	-	-	25.1	[29]
6	2010	Karachi (peri-Urban)	EIA[4]	649	1348	1997	19	26	23.83	[28]
7	2010	Karachi	ICT[9]	351	153	504	2.8	5.23	3.17	[34]
8	2010		ELISA[9]							[41]
		Multan		625	-	625	9.6	-	9.6	
		Lahore		1892	-	1892	9.4	-	9.4	
		Faisalabad		2736	-	2736	8.8	-	8.8	
		Gujranwala		16522	-	16522	7.3	-	7.3	
		Gujrat		9770	-	9770	6.8	-	6.8	
		Sargodha		1620	-	1620	6.7	-	6.7	
		Rawalpindi		445	-	445	6.7	-	6.7	
		Sialkot		24707	-	24707	6.2	-	6.2	
		Bahawalpur		363	-	363	5.0	-	5.0	
9	2010	Islamabad	ICT[9]	-	252	252	-	24.6	24.6	[40]
10	2010	Mansehra	ICT[9]	394	254	648	11.8	9.4	10.34	[44]
11	2010	National Survey	ICT[3]	24444	22599	47043	4.9	4.8	4.87	[21]
12	2011	Gujranwala	EIA[5]	1770	732	2502	2.6	1.68	2.32	[43]
13	2011	Karachi	CLIA[6]	-	-	32049	-	-	9.75	[35]
14	2012	Kech	EIA[7]	709	1291	2000	7.62	4.34	5.5	[26]
15	2012	Punjab	EIA[7]	14027	14027	-	3.13	-	3.13	[39]
16	2014	Mardan	ICT[8]	757	662	1419	13.6	9.52	11.7	[37]
17	2014	Peshawar	CMIA[4]	543	439	982	15.4	9.7	12.93	[42]

[1]Nobis Labordiagnostica, Cluj-Napoca, Romania; [2]Acon Laboratories, CA, United States; [3]Bionike Inc. CA, United States; [4]Abbott Diagnostics, IL, United States; [5]DSI, srl. Saronno VA, Italy; [6]Roche Diagnostics, CA, United States; [7]DiaSorin International Inc., Saluggia, Italy; [8]Accurate Diagnostic Labs, NJ, United States; [9]Manufacturer not mentioned in cited reference. ELISA: Enzyme linked immunosorbent assay; CLIA: Chemiluminescence immunoassay; ICT: Immunochromatographic test; EIA: Enzyme immunoassay; CMIA: Chemiluminescent microparticle immunoassay.

the last two decades, from 1994 to 2015 (Figure 1). All studies that investigated HCV prevalence in the general adult population were included. We found fifty relevant studies[21,26,28,29,34-48,50-80] published in international and local journals. In total 366066 individuals were screened in these studies. Reports from almost all provinces/administrative units of Pakistan were included. Moreover, a detailed district-wise prevalence estimate was available through the results of the national survey. Where more than one report was available from same district, a weighted mean was calculated using the data (including the national survey). For districts for which no reports, other than the national survey were available, the data was used to construct the district wise prevalence map which is presented in Figure 1.

HCV seroprevalence in blood donors

Regular screening of blood donors is essential for controlling the spread of transfusion transmitted infections (TTIs). Screening of blood for TTIs, including HCV, prior to transfusion is now a common practice among healthcare providers in Pakistan, especially after the introduction of a comprehensive National Blood Policy in 2003 and development and implementation of blood transfusion laws in all the provinces and units of federation[81]. However, the risk of non-implementation and non-adherence to these regulations is still quite high, particularly in remote and underdeveloped areas[16]. Thirteen studies[25,27,82-92] published between 2009 and 2014 reported HCV prevalence in blood donors (Table 2). Mean HCV prevalence among healthy blood donors was estimated at 2.45%. A majority of blood donors hailed from the country's largest city Karachi alone (159942 out of total of 400716)[83,86,87,90], where mean prevalence of HCV infection among blood donors was 2.31%. Pakistan's largest province Punjab remained underrepresented in this group, as only three reports comprising of 10345 patients were available[82,85,88]. A study conducted by Akhtar *et al*[82] reported a very high prevalence (15.1%) of HCV infection among blood donors from Lahore, the second largest metropolitan city in Pakistan. Khan *et al*[27] reported a 20.8% seroprevalence of HCV infection in a group of 356 blood donors hailing from Quetta, capital of Balochistan, the province which is otherwise considered a low endemicity area (1.5% prevalence in national survey)[21].

HCV infection in patients with liver disease

Hepatitis C virus infection is an important underlying cause of liver disease and accounts for about 25% of

Table 2 Seroprevalence of hepatitis C virus infection in blood donors

S. #	Year	Place	Method	Sample size	Seroprevalence (%)	Ref.
1	2009	Mirpurkhas	ELISA[7]	804	15.05	[25]
2	2010	Multan	ELISA[1]	10000	4.90	[88]
3	2010	Peshawar	ELISA[7]	32042	1.57	[92]
4	2011	KPK/FATA	ELISA[2]	7148	1.89	[89]
5	2011	KPK/FATA	ELISA[2]	62251	2.60	[91]
6	2011	Karachi	-[8]	5717	1.90	[86]
7	2012	Karachi	ELISA[3]	5517	2.00	[83]
8	2012	Peshawar	ELISA[2]	127828	2.46	[84]
9	2012	Sargodha	ELISA[3]	100	12.00	[85]
10	2013	Karachi	MEIA/CLIA[4]	108598	2.61	[87]
11	2013	Quetta	ELISA[2]	356	20.8	[27]
12	2013	Lahore	ELISA[5]	245	15.00	[82]
13	2014	Karachi	CLIA[6]	42830	1.65	[90]

[1]Labkit Chemelex, Barcelona, Spain; [2]Biokit; [3]General Biologicals Corporation, Taiwan; [4]Abbott Diagnostics, [5]DiaSorin; [6]Ortho Clinical Diagnostics, NJ, United States; [7]Manufacturer not mentioned in cited reference; [8]Method not mentioned in cited reference. KPK: Khyber Pakhtunkhwa; FATA: Federally Administered Tribal Areas; ELISA: Enzyme linked immunosorbent assay; MEIA: Microparticle immunoassay; CLIA: Chemiluminescence immunoassay.

Table 3 Hepatitis C virus in patients with liver disease

S. #	Year	Place	Method	Sample size	Seroprevalence (%)				Ref.
Hepatocellular carcinoma									
1	2009	Punjab/KPK	ELISA[1]	145	76.5				[97]
2	2009	Pakistan	EIA[2]	82	79.2				[98]
3	2010	Karachi	-[5]	40	50				[94]
4	2013	Pakistan	ELISA[4]	645	48.5				[95]
5	2014	Hyderabad	ELISA[4]	188	66				[96]
Liver disease					AH	CH/CLD	Carrier	Cirrhosis	
6	2009	Swat	ELISA[4]	110	-	-	-	63.6	[99]
7	2009	Pakistan	EIA[2]	107	-	62.6	-	24.3	[98]
8	2010	Karachi	-[5]	5153	7	72.9	51.7	59.4	[94]
Hepatic encephalopathy									
9	2009	Hyderabad	-[5]	87	66.67				[100]
Suspected for viral hepatitis									
10	2014	Islamabad	ELISA[3]	845	24.8				[101]

[1]DRG Instruments, Germany; [2]Abbott Diagnostics; [3]Biokit; [4]Manufacturer not mentioned in cited reference; [5]Method not mentioned in cited reference. AH: Acute hepatitis; CH: Chronic hepatitis; CLD: Chronic liver disease; ELISA: Enzyme linked immunosorbent assay; EIA: Enzyme immunoassay; KPK: Khyber Pakhtunkhwa.

hepatocellular carcinoma (HCC) cases worldwide[93]. However, epidemiological data suggests that in contrast to the worldwide trend, where HBV is considered the major etiology underlying HCC, in countries of high HCV prevalence such as Egypt and Pakistan, HCV is the most common cause of chronic liver diseases including HCC[93]. A previous comprehensive review of all the studies published before 2010 (ten studies) estimated 50.6% mean frequency of HCV seropositivity among HCC patients in Pakistan[23]. Three studies published between 2010 and 2014[94-96], and also two studies published in 2009[97,98] but not included in previous reviews[16,23,24], dealing with the frequency of HCV infection HCC patients were identified. These studies screened 1100 HCC patients from different areas of Pakistan. Serofrequency of HCV infection among HCC patients ranged from 48.5%[95] to 79.2%[98] (Table 3, entries 1-5). Mean HCV prevalence in this patient group was 57.5%.

Three studies reported HCV prevalence among cirrhosis patients[94,98,99] (Table 3, entries 6-8). Khan and colleagues found 24.3% cirrhotic patients positive for anti-HCV[98], another report from a different group showed that 63.6% cirrhotic patients in the district Swat were infected with HCV[99]. Ahmed and coworkers found 59.4% HCV seroprevalence among cirrhotic patients in Karachi city[94]. Similarly Khan *et al*[98] and Ahmed *et al*[94] found 62.6% and 72.9%, respectively, HCV seroprevalence among patients suffering from chronic hepatitis or chronic liver disease. A hospital based study conducted by Devrajani *et al*[100] in 2009 found that more than two thirds of patients reporting with complaint of hepatic encephalopathy tested positive for the presence of anti-HCV in their blood. A recent single center study showed that among all the patients referred to the said hospital with a suspected hepatitis virus infection, approximately one quarter were found to be positive for HCV[101] (for details see Table 3).

Table 4 Hepatitis C virus serofrequency in high risk population groups

S. #	Year	Place	Method	Sample size	Seroprevalence (%)	Ref.
Pregnant women						
1	2009	Hazara	ELISA[1]	500	8.6	[104]
2	2009	Swat	ICT[6]	5607	2.6	[105]
3	2009	Karachi	EIA[2]	5902	1.8	[106]
4	2010	Multan	ICT[6]	500	7.0	[107]
5	2011	Karachi	ELISA[3]	18000	5.79	[102]
6	2013	Hyderabad	ELISA[2]	3078	4.7	[103]
Multi-transfused population (including pediatric population)						
7	2009	Islamabad	ELISA[6]	103	36.0	[108]
8	2010	Lahore	Questionnaire	408	1.5	[110]
9	2011	KPK	ICT[4]	40	15.0	[111]
10	2011	Karachi	ELISA[3]	173	51.4	[86]
11	2012	Karachi	ELISA[5]	160	13.0	[83]
12	2014	Rawalpindi	ELISA[6]	95	49.5	[109]
Intravenous drug users						
13	2011	KPK	ICT[4]	42	14.28	[111]
14	2011	KPK	ICT[4]			[113]
		Peshawar		100	35.0	
		Kohat		60	25.0	
		Mardan		40	32.5	

[1]Ortho Clinical Diagnostics; [2]Abbott Diagnostics; [3]DiaSorin; [4]Acon Laboratories, CA, United States; [5]General Biologicals Corporation; [6]Manufacturer not mentioned in cited reference. KPK: Khyber Pakhtunkhwa; ELISA: Enzyme linked immunosorbent assay; EIA: Enzyme immunoassay; ICT: Immunochromatographic test.

PREVALENCE OF HCV IN HIGH RISK POPULATION GROUPS

Pregnant women

It has previously been suggested that the predominant mode of HCV transmission in Pakistan is nosocomial or iatrogenic[16]. Therefore pregnant women who require more hospital visits and have to undergo more medical and surgical procedures are considered to be at an increased risk of contracting HCV infection. Based on data from six relevant studies[102-107], mean HCV prevalence in pregnant women was estimated around 4.51%. The prevalence of HCV infection in pregnant women ranged from 1.83% in Karachi[106] to 8.6% in Hazara division[104] (Table 4, entries 1-6).

Multi-transfused individuals

Patients suffering from congenital coagulation disorders, or other diseases that require multiple transfusions of blood at regular intervals throughout their life, are also considered at an increased risk of catching transfusion transmitted infections including HCV. Six relevant studies, covering 979 patients that were regular recipients of blood or blood products transfusions, were identified (Table 4, entries 7-12)[83,86,108-111]. The results obtained were surprisingly variable. HCV prevalence rates in this group ranged from as low as 1.47% in Lahore[110] to around 50% at centers located in Rawalpindi and Karachi[86,109]. Mean HCV prevalence in multi-transfused patients was calculated as 21.04%.

Intravenous drug users

Intravenous drug use is one of the most important risk factors in HCV transmission worldwide[1]. However, its relative contribution to the HCV endemic in Pakistan, is not clear. According to a recent United Nations Office on Drugs and Crime report, some 6-7 million Pakistanis admitted using drugs in the past one year, around half million of which were regular intravenous drug users (IDUs)[112]. Unfortunately, not many studies are available which have focused on serofrequency of HCV infection in this high risk group. Only two recent studies reported HCV prevalence in various districts of KPK province[111,113]. These reports show a prevalence of HCV infection among IDUs to range from 14.3% to 35%. The results of these studies are summarized in Table 4 (entries 13 and 14).

Health care workers

Risk of HCV transmission in health care workers (HCWs) is particularly high[114], and regular screening of this population subgroup is important in order to curtail further spread of HCV infection to patients. Unfortunately not many relevant studies could be found. Only two studies published in the recent past determined anti-HCV frequency in HCWs and both originated from KPK province (Table 5, entries 1 and 2)[115,116]. Khan *et al*[115] screened HCWs from three major hospitals in Peshawar and reported 4.1% prevalence of HCV, while Sarwar *et al*[116] reported 5.6% HCV prevalence among HCWs at various hospitals of Abbottabad. An earlier review of published data suggested similar levels of HCV prevalence in HCWs, except for those who reported a history of needle stick injury, in which the prevalence was as high as 10%[24]. Based on available data it is likely that HCV prevalence in HCWs in Pakistan is equal to that of general population.

Table 5 Hepatitis C virus serofrequency in miscellaneous high risk population groups

S. #	Year	Place	Method	Sample Size	Seroprevalence (%)	Ref.
Health care workers						
1	2008	Abbottabad	ELISA[7]	125	5.6	[116]
2	2011	Peshawar	ICT[1]	824	4.1	[115]
Prisoners						
3	2010	Sindh	ELISA[2]	7539	12.8	[117]
4	2010	Karachi	ELISA[3]	357	15.2	[118]
Dialysis patients						
5	2011	KPK	ICT[4]	25	28.0	[111]
6	2011	Peshawar	ELISA[1]	384	29.2	[119]
Homosexual community						
7	2010	Sindh	ELISA[5]	396	23.5	[120]
Vertical transmission						
8	2011	Karachi	ELISA[6]	129	3.9	[102]

[1]Acurate Diagnostics; [2]Medical Biological Services, Italy; [3]Abbott Diagnostics; [4]Acon Laboratories; [5]Biokit; [6]DiaSorin; [7]Manufacturer not mentioned in cited reference. KPK: Khyber Pakhtunkhwa; ELISA: Enzyme linked immunosorbent assay; ICT: Immunochromatographic test.

Other high risk groups

Other population subsets are also considered at increased risk of HCV infection (Table 5). These include prison inmates, patients on regular dialysis, as well as the homosexual community. Two reports published in 2010 found the serofrequency of HCV infection among inmates of various prisons in Sindh province to be 12.8% and 18.2% (entries 3 and 4 in Table 5)[117,118]. Among dialysis patients, 28%-29% tested positive for presence of HCV antibodies in their blood (entries 5 and 6 in Table 5)[111,119]. Khanani and colleagues reported that 23.5% of 396 homosexuals/MSMs (males who have sex with males) tested positive for anti-HCV (entry 7, Table 5)[120]. In addition, Aziz *et al*[102] reported a 4% rate of vertical HCV transmission from infected mothers to newborn children of ages up to 18 mo (entry 8, Table 5).

Patients seeking hospital care

Screening of patients suffering from diseases, which are not considered a direct consequence of HCV infection or do not directly pose an increased risk of contracting HCV infection, except for the risk of nosocomial and iatrogenic transmission, is also an important surveillance strategy. Firstly, it can provide a window into the prevalence in general population and secondly, for an infection like HCV, it can also provide useful insights into the possible risk factors involved in the transmission of this virus. At least thirteen studies reported the prevalence of HCV in patients (Table 6)[88,106,111,121-130]. These patients visited/attended hospitals with various complaints ranging from general mild sickness requiring outpatient care, to major and gynecological surgeries as well as dermatological and urological disorders.

The results show an alarmingly high rate of HCV prevalence. 14.2% and 16.2% prevalence in patients undergoing dental[111] and gynecological surgeries[106] respectively, clearly points towards a possible high rate of hospital acquired infection. With the exception of the study by Majid *et al*[125], which showed a 3.3% prevalence in patients at a single hospital in district Bannu, most studies reported prevalence rates much higher than the national prevalence estimated here (6.8%) as well as earlier systematic reviews[16,23,24] and the national survey[21]. A recent report from Faisalabad indicated alarmingly high HCV seropositivity (21.99%) among patients visiting a sexually transmitted infections clinic[124].

COMPARISON OF METHODS USED IN SEROPREVALENCE STUDIES

A variety of methods have been used for the diagnosis of HCV infection. Studies cited in this review used methods ranging from rapid, point of care tests, such as the ICT, to more expensive laboratory conducted assays such as ELISA, EIA, CMIA and CLIA. A recent meta-analysis of at least 30 different studies that compared point of care testing results with those of more advanced laboratory based assays used for HCV diagnosis showed a high pooled accuracy for all studies[131]. However, the same study showed a high degree of heterogeneity in performance of various commercially available point of care tests. Caution therefore must be observed while selecting appropriate rapid HCV diagnosis testing platforms. While it is beyond the scope of this article to recommend certain manufacturer(s) over others, readers are referred to the WHO list of prequalified diagnostics[132] or other studies reporting a direct comparison of different commercially available HCV diagnostic tests (*e.g.*[133]). Furthermore, although rapid tests like ICT are quite efficient in diagnosing HCV infection with less than 1% false negative rates, such screening tests should not be considered as sole diagnostic criteria, as studies have reported high rate of false positive results[38]. Finally considering the fact that some 15%-45% HCV infected patients may spontaneously clear the virus but still remain seropositive for HCV[30,31], as discussed

Table 6 Hepatitis C virus seropositivity among patients other than those with liver disease

S. #	Year	Place	Method	Sample size	Seroprevalence (%)	Ref.
Urological patients						
1	2010	Lahore	ELISA[4]	558	13.4	[130]
Major gynecological surgery						
2	2009	Karachi	EIA[1]	548	16.2	[106]
Surgery (eye/elective/major)						
3	2010	Sukkur	ICT[5]	913	13.8	[126]
4	2011	KPK	ICT[2]	25	8.0	[111]
5	2013	Kharian	ELISA[5]	554	6.4	[122]
Dental surgery						
6	2011	KPK	ICT[2]	35	14.28	[111]
Type II diabetes						
7	2010	Multan	ELISA[3]	3000	30.2	[88]
Cataract patients						
8	2012	Karachi	ELISA[5]	377	11.4	[127]
Dermatological disorders						
9	2012	Karachi/ Rawalpindi	ELISA[5]	355	9.01	[128]
Tuberculosis patients						
10	2013	Rahim Yar Khan	ELISA[4]	110	9.1	[121]
Sexually transmitted infection patients						
11	2014	Faisalabad	ELISA[1]	39780	21.99	[124]
Seeking hospital care (Misc diseases/disease not defined)						
12	2010	Bannu	ELISA[5]	25944	3.3	[125]
13	2011	Karachi	ELISA[1]	2965	12.8	[123]
14	2011	Kotli (AJK)	ELISA[5]	9564	6.38	[129]

[1]Abbott Diagnostics; [2]Acon Laboratories; [3]Labkit; [4]Bio-tech Company Limited, United States; [5]Manufacturer not mentioned in cited reference. KPK: Khyber Pakhtunkhwa; ELISA: Enzyme linked immunosorbent assay; EIA: Enzyme immunoassay; ICT: Immunochromatographic test.

in preceding paragraphs, a positive anti-HCV test should be followed by nucleic acid testing as per WHO and CDC guidelines[32].

PREVALENCE OF ACTIVE HCV INFECTION

Recent WHO and CDC guidelines recommend NAT directly following a positive anti-HCV test[32], so that an active HCV infection can be differentiated from a false positive or resolved past infection. However, not much attention has been paid to distinguishing active HCV infection from seroprevalence of anti-HCV. Only five studies (Table 7) reported active HCV infection[36,37,45,134,135]. These studies encompassed a combined samples size of 7158 persons. Based on these studies, active HCV infection was found to be approximately 6% in the general population of Pakistan. Active HCV infection ranged from 3.5% in Mansehra[36] to 17.2% in Rawalpindi[135] (Table 7, entries 2 and 3, respectively).

Similarly, only two reports have been published on active HCV infection among IDUs in Pakistan since 2010[111,113]. While Ali and colleagues found 14.3% active HCV infection among a small group of 42 IDUs[111], a study by Rehman *et al*[113] reported a very high (24%) HCV prevalence among 200 IDUs. Active HCV infection has not been adequately investigated in other population subsets, particularly the high risk groups. Except for dialysis patients, for which two reports are available[111,119], only one report each was published for blood donors[89], health care workers[115], pregnant women[102], multi-transfused patients[111] and patients undergoing major surgery[111]. The results of each of these studies are detailed in Table 7 and show an alarmingly high rate of active HCV infection.

FREQUENCY DISTRIBUTION OF HCV GENOTYPES IN PAKISTAN

Methodologies used for genotyping of HCV isolates

HCV genotype is an important determinant of disease severity and pathogenesis as well as patient response to antiviral therapy. Therefore accurate genotyping of HCV isolates is of fundamental significance. Several HCV genotyping approaches have been reported in the literature and have been reviewed comprehensively elsewhere[136]. Readers are referred to aforementioned review as well as other recent studies (*e.g.*[137,138]) in order to develop a better understanding of advantages and shortcomings related to each methodology. Studies included in this review mostly relied on PCR based amplification of HCV Core and/or 5' non-coding region sequences using subtype specific primers. The amplicons thus generated differ in their sizes and genotype/subtype of an isolate can be determined by gel electrophoresis[13,139]. The method originally developed by Ohno and coworkers[13] has been extensively used and most of the studies included in this review[17,18,37,45,115,119,140-149] used this methodology.

Table 7 Prevalence of active hepatitis C virus infection in Pakistan

S. #	Year	Place	Sample size			Active HCV infection (%)			Ref.
			Male	Female	Total	Male	Female	Total	
General population									
1	2010	Swat	290	300	590	2.8	5.7	4.2	[45]
2	2010	Mansehra	300	100	400	4.0	2.0	3.5	[36]
3	2012	Rawalpindi	147	156	303	17.7	16.7	17.2	[135]
		Islamabad	75	125	200	5.3	3.2	4.0	
4	2013	Lahore	1914	2332	4246	5.3	4.7	4.9	[134]
5	2014	Mardan	757	662	1419	11.5	5.1	8.5	[37]
Intravenous drug users									
6	2011	KPK			42			14.3	[111]
7	2011	KPK			200			24.0	[113]
Dialysis patients									
8	2011	KPK			25			28.0	[111]
9	2011	KPK			384			27.6	[119]
Blood donors									
10	2011	KPK/FATA	-	-	7148	-	-	1.6	[89]
Health care workers									
11	2011	KPK			824			2.8	[115]
Multi transfused population									
12	2011	KPK			40			15.0	[111]
Patients undergoing major surgery									
13	2011	KPK			25			8.0	[111]
Dental surgery patients									
14	2011	KPK			35			14.3	[111]
Pregnant women									
15	2011	Karachi			18000			-[1]	[102]

[1]Out of 18000 subjects studied, 1043 women tested positive for anti- hepatitis C virus (HCV) during screening; only 640 out of these 1043 agreed to undergo nucleic acid testing of which 510 tested positive for HCV-RNA. KPK: Khyber Pakhtunkhwa; FATA: Federally Administered Tribal Areas.

This methodology was further refined by Idrees in 2008[139] by designing primers based on updated HCV genome sequence data with particular emphasis on isolates from Pakistan. A few of the studies relied on the method developed by Idrees[113,150-154]. Very few studies reported using commercially available genotyping assays. For example Ahmad *et al*[155] and Ijaz *et al*[156] used the Invader HCV Genotyping Assay (Third Wave Technologies, Inc., WI, United States) while Akhund and coworkers used a commercially available, type specific PCR amplification kit (AnaGen Technologies Inc., GA, United States)[157].

Frequency distribution of HCV genotypes

Genotyping of HCV isolates from Pakistan has been carried out extensively in the recent past. Twenty-five reports were published between 2010 and 2014 that pertained to the distribution of various HCV genotypes in Pakistan[17,18,37,45,113,115,119,141-158]. However, none of the studies reported the genotype distribution in Balochistan province. Therefore, so as to present a more comprehensive picture of HCV genotype frequency distribution in Pakistan, a 2009 study pertaining to Balochistan province was included[140] (Table 8). A total of 37025 subjects were genotyped in these studies and show a heterogeneous pattern of genotype distribution in various regions of Pakistan. Genotype 3a was found in approximately 61.4% patients as mono-infection, as well as in most of the patients with mixed genotype infection.

Overall, approximately 12.5% samples could not be assigned a specific genotype or subtype by the methods used, making the proportion of untypable samples higher than all the subtypes except 3a. A province wise break-up of untypable samples revealed the highest levels in samples originating from Balochistan (32.1%) and KPK province (12.6%). Only 2.7% of samples from Punjab could not be typed. It is worthwhile discussing here the diagnostic and therapeutic relevance of a high ratio of untypable samples. Due to the high rate of mutations in the HCV genome, as well as other factors mentioned by Afzal *et al*[158], the emergence of new subtypes or genetic variants that are untypable by current methods is unavoidable. There is an urgent need not only to upgrade genotyping methodologies by using updated HCV sequence information, but also to develop a consensus reference genotyping method in order to avoid cross-methodology ambiguities. Sequencing of untypable samples can be of great importance in this regard, as it will not only help researchers to upgrade methodologies but might also help in identifying new genotypes/subtypes.

A closer look at the frequency distribution of genotypes other than 3a also indicated a change in the distribution pattern of HCV genotypes in Pakistan. Overall, genotype 3 (69.1%) was the most prevalent genotype in Pakistan, followed by genotypes 1 (7.1%),

Table 8 Frequency distribution of hepatitis C virus genotypes in Pakistan

Frequency (%) of hepatitis C virus genotypes																		Ref.
1			2			3			4			5		6		Mixed	Untypable	
1a	1b	U	2a	2b	U	3a	3b	U	4a	4b	U	5a	U	6a	U			
3.5	0.8	-	1	-	-	88.1	3	-	-	-	-	-	-	-	-	3.6	-	[142]
6.1	6	-	15.1	6.1	-	42.3	12.1	-	-	-	-	-	-	-	-	-	12.1	[45]
0.72	0.72					80.26	6									6.73	27.88	[151]
5.4			8.1			34.1	7									7.6	37.8	[154]
1.5	2.5		39	1		31	8										17	[17]
7.14			35.71			28.6					14.29						14.29	[113]
12.1	1.2	-	1.2	0.4	0.3[1]	64.5	6.4	0.001[1]	6.7	0.6	-	0.5	-	0.4	-	3.2	2.4	[156]
14.3	3.6	-	8.9	1.8	-	32.1	17.8		1.8							10.7	8.9	[119]
6.8	4.6		1.3			54.4	8.2									8.2	16.4	[152]
5.6	-	-	-	-	-	90.3	0.6	-	-	-	0.6	-	-	-	-	2.8	-	[141]
2.6	0.8	-	0.3	0.2	-	82.6	0.2	-	-	-	-	0.1	-	-	-	2.4	10.8	[150]
12.5	8.9		9.8	1.8		42.9	22.3										1.8	[149]
7.4	5.8	-	13.2	6.6	-	26.4	16.5	-	-	-	2.5	-	-	-	-	4.1	17.4	[37]
10	3.6	-	-	-	-	56.4	15	-	-	-	1.4	-	-	-	-	7.1	6.4	[143]
1.3	0.7	-	-	-	-	82.1	13.9	-	-	-	-	-	-	-	-	2	-	[146]
1	-	-	12	-	-	38.2	21	-	-	-	-	-	-	-	-	7.3	21	[148]
8.8	3	-	6.5	1	-	45.5	16	-	0.8	-	-	-	-	-	-	16	2.2	[145]
5.3	5.1	1[1]	24.9	6.1	-	39.4	6.3	-	2.4			2.4		2		-	5.7	[18]
2.9	1.5	-	-	-	1.2	70.3	5.5	-	-	-	-	-	0.9	-	-	2.6	15.1	[157]
3.3	0.8	0.2[1]	2.1	0.2	0.02[1]	61	8.9	0.24[1]	-	-	0.5	0.1		0.1		4.7	17.8	[153]
23.6	-	-	-	-	-	55.9	3.2	-	12.5	1.2	-	-	-	-	-	1.2	2.5	[155]
4.3	-	-	-	-	-	73.9	13	-	-	-	-	-	-	-	-	4.3	4.3	[115]
0.9	0.9	-	7.4	0.9	-	66.1	2.6	-	-	-	-	-	-	-	-	2.2	18.8	[144]
-	-	-	1.1	2.2	-	86.8	2.2	-	-	-	-	-	-	-	-	-	7.7	[149]
7.1	-	-	-	-	-	50	10.7	-	-	-	-	-	-	-	-	-	32.1	[140]
0.5	1.5	-	10.3	1.2	-	68.3	2.6	-	-	-	-	-	-	-	-	-	15.6	[158]

[1]Subtype C. U: Undefined subtype.

2 (4.2%) and 4 (2.2%). Genotypes 5 and 6 both accounted for approximately 0.2% each of the total sample pool and thus are rare in Pakistan. Samples with mixed genotype accounted for 4.2% of total samples. Among subtypes, overall genotype 3a was found in 61.4% of the samples, followed by genotype 3b (7.6%), 1a (5.7%), 2a (3.7%) and 1b (1.4%). The frequency of all the other subtypes (1c, 2b, 2c, 3c, 4b, 5a and 6a) was found to be less than 1% each.

The genotype distribution in each province showed important differences (Figure 2). A higher frequency of genotype 2 was observed in Sindh and KPK province (11.3% and 17.3% respectively), making it the second most prevalent typable genotype in these provinces. The relative frequency distribution of various subtypes differed considerably across Pakistan. Although genotype 3a was found to be the most prevalent subtype in all three provinces for which reports were available (67.7%, 53.9% and 46.9% in Punjab, Sindh and KPK, respectively), genotype 2a emerged as the second most prevalent subtype in typable samples originating from Sindh and KPK provinces (6.06% and 15.1% respectively). In contrast, only 1.9% of samples from Punjab province tested positive for genotype 2a and the second most prevalent subtype after 3a was found to be 1a (10.8%) in this province. Overall the frequency distribution of all subtypes found is shown in Figure 3 while Figure 2 shows a province wise break-down of major HCV genotype distributions. Results from individual studies are summarized in Table 8.

CONCLUSION

HCV prevalence data published recently (2009/10 to 2015) suggests that HCV infection is on the rise in Pakistan. While the almost 40% increase in HCV seroprevalence among the general population suggested by the analysis here, compared to previous estimates (6.8% rather than 4.7%-5%) is alarming, high prevalence rates among persons who have had surgical and medical interventions suggest a predominant involvement of nosocomial transmission in the spread of HCV in Pakistan. Given the evidence of high nosocomial transmission as well as increased burden of this disease in lesser developed areas (discussed below) where health service related malpractices are more common, there is an urgent need to implement strict measures in order to ensure safe medical practices. Reducing unnecessary injections, ensuring that surgical and dental instruments are sterilized or disposable instruments are used, avoiding shared razors at barber shops as well as unhygienic piercing and tattooing instruments are among the most important preventive measures. Although the response rate to conventional IFN-ribavirin therapy regimen among genotype 3a patients (the most prevalent genotype in Pakistan) is

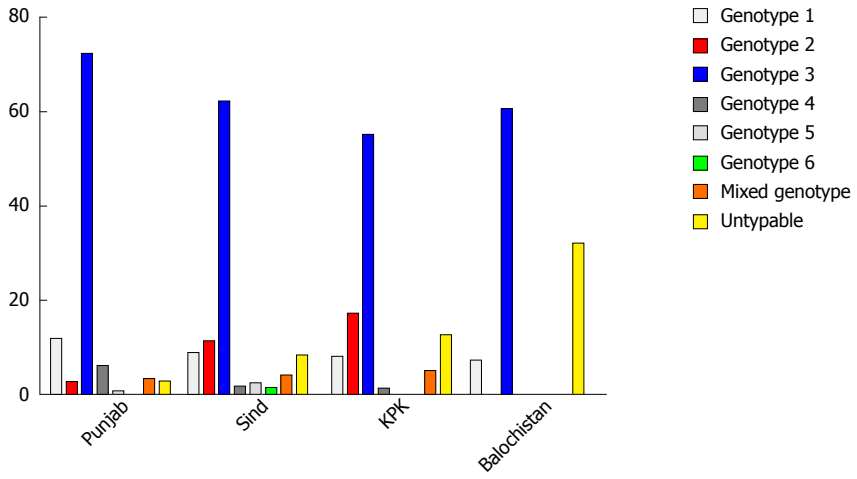

Figure 2 Distribution pattern of major hepatitis C virus genotypes at the province level. KPK: Khyber Pakhtunkhwa.

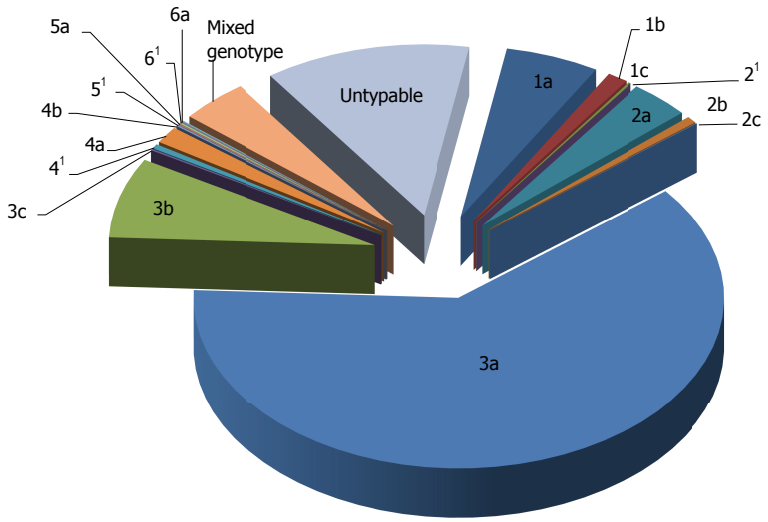

Figure 3 Relative frequency distribution of hepatitis C virus subtypes in Pakistan. [1]Undefined subtype.

already quite good and with the availability of direct acting antivirals such as Sofosbuvir at reduced prices for patents in Pakistan, it is hoped that HCV can be controlled more effectively.

Reports reviewed in the current study also suggest that there is extremely high HCV prevalence in under-developed rural and peri-urban areas. However, much less attention has been paid to this socioeconomic dimension of the HCV epidemic in Pakistan. Considering the fact that a majority of the population of Pakistan resides in these rural areas with high HCV prevalence, it is likely that the actual burden of hepatitis C in Pakistan may be much higher. Undoubtedly, an increased focus is needed to gauge HCV prevalence in rural areas for better evaluation and implementation of preventative strategies. Moreover, in light of recent WHO and CDC guidelines, it is imperative that more effort is invested in determining the prevalence of active HCV infection in Pakistan.

The analysis also showed a shift in relative frequency distribution of HCV subtypes in various regions of Pakistan. Although 3a continues to be the most prevalent genotype, recent years have witnessed an increase in incidence of genotype 2a infection in KPK and Sindh provinces. This temporal change in relative distribution of various genotypes has fundamental implications with regard to translational efforts aimed at limiting and eradicating HCV in Pakistan. The high proportion of samples that could not be assigned to a specific genotype demands improvements in methodologies currently used in order to develop a better understanding of HCV genotype distribution and evolutionary trends in Pakistan.

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