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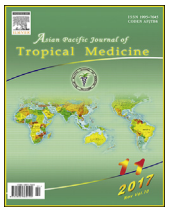

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Pattern of hepatitis C virus genotypes and subtypes circulating in war-stricken Khyber Pakhtunkhwa, Pakistan: Review of published literature

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ABSTRACT

Infection due to hepatitis C virus (HCV) is a major cause of fibrosis and hepatocellular carcinoma in Pakistan. In the current review, pattern of HCV genotypes and subtypes in Khyber Pakhtunkhwa province was ascertained in light of the available literature. After thorough analysis, genotype 3 (58.27%) was determined to be the leading HCV genotype, followed by genotypes 2 (12.39%), 1 (9.54%) and 4 (0.86%). The proportions of genotypes 5 and 6 were recorded as 0.09% and 0.22% respectively. Subtype wise, 3a accounted for 48.67%, followed by subtype 2a (10.91%), 3b (9.43%), 1a (5.84%), 1b (3.66%), 2b (1.45%) and genotype 4 with its undefined subtypes contributed a portion of 0.86%. The cumulative share of subtypes 1c, 2c, 3c, 5a and 6a was less than 1%. In 11.51% cases, the subtype was untypeable while in 7.17% cases mixed subtypes were recorded. Gender wise, proportions of most HCV subtypes were marginally higher among males as compared to females. On the basis of studied groups, 3a was pervasive among all groups except in intravenous drug users where 2a was the major HCV subtype. Similarly, based on various geographical locations (provincial divisions), subtype 3a revealed a ubiquitous distribution. Conclusively, HCV 3a persists to be the principal subtype across the province of Khyber Pakhtunkhwa. The considerable number of untypeable subtypes in most studies urges for an improved genotyping system on the basis of local sequence data and practice of sequencing for determination of underlying subtype in untypeable cases. Further, studies on identification of subtypes transmission pattern are imperative for assessment of transmission origin and reinforcement of efficient control strategies. In addition, the current review emphasizes the need of attention toward HCV risk groups and ignored southern side of Khyber Pakhtunkhwa province for better holistic understanding of HCV genotype distribution pattern in the province.

1. Introduction

Hepatitis C is a serious health menace caused by hepatitis C virus (HCV). Worldwide, about 180 million people are affected by HCV with an annual death toll of 350 000 due to hepatitis C-associated liver complexities [1]. In South Asia, approximately 50 million people retain HCV [2]. Pakistan is one of the high burden countries with 10 million chronically infected HCV carriers [3].

HCV is a hepatotropic virus that belongs to genus *Hepacivirus* of family Flaviviridae. Genome of the virus is a single stranded sense RNA of 9.6 kb. HCV genome encodes three structural and seven non-structural genes. Due to RNA nature and lack of proofreading mechanism, the viral RNA-dependent-RNA-polymerase gives rise to the formation of genetic variants during replication [4]. Previously, HCV has been classified into six major genotypes and numerous subtypes. Worldwide, HCV genotypes and subtypes vary in their geographical distribution. Genotype 1, 2 and 3 occur globally, genotype 4 is widespread in Middle East and Africa, genotype 5 is prevalent in South Africa while genotype 6 in South-east Asia [2,3]. In Pakistan, genotype 3 is the most pervasive genotype while 3a is the frequently occurring HCV subtype [5].

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Studies on determination of HCV genotypes are helpful in assessment of regional infection epidemiology and genotypes distribution pattern. HCV genotypes may vary considerably in different countries and within different regions of the same country [6]. Khyber Pakhtunkhwa (KP) province is located in the northern corner of Pakistan. Due to front line province in war against insurgency for more than a decade and consecutive natural disasters, the province remain neglected in mainstream studies focusing health and epidemiology. Further, premises of KP shares porous border with the politically chaotic Afghanistan and backward territory of Federally Administered Tribal Areas. Therefore, health and disease dynamics are influenced by influx of immigrants and external populations, harboring the province a distinct status from rest of the country. Previously, a review study focusing HCV genotypes and subtypes examined overall situation of the country [7]. However, no dedicated genotype assessment review study exists to highlight HCV carriers in KP exclusively. Moreover, a recent study indicated shift in the HCV subtype distribution pattern in KP [8]. Therefore, the current study was devised to get a detailed overview of HCV genotypes and subtypes distribution pattern in this previously ignored part of Pakistan.

2. Material and methods

2.1. Literature survey

A literature survey was conducted in November 2016 in search of articles related to distribution, occurrence and epidemiology of HCV genotypes in KP. Popular electronic databases Google Scholar and PubMed were explored for scientific articles published during the year 2005 till first half of 2016. To get a comprehensive pool of relevant articles, end references were also utilized for retrieval of useful papers. After scrutiny, original papers containing matching information with the study objectives were picked up. In years 2005, 2006, 2007 and 2009 no published study material was identified. Overall, nineteen articles [3,5,8–24] from various geographical sites of the province were included for analysis in the final selection (Table 1). The retrieved data was carefully arranged into categories.

2.2. Statistical analysis

SPSS software was used for assessment of relative percentage and statistical significance. The *t*-test (two-tailed) was applied to ascertain the association of HCV subtypes distribution with gender at 0.05 level of significance (*P* value).

3. Results

3.1. Genotyping assays

Globally, HCV genotyping is carried out with various methods including type-specific PCR, restriction fragment length polymorphism (RFLP), line probe hybridization assay (LiPA) and genome sequencing [25]. In KP, type-specific PCR is the most widely-used approach for HCV genotyping. In this review, all the studies (Table 1) utilized type-specific amplification of HCV core and/or 5′ un-translated region for specific size PCR amplicons generation. Generally, two methods of HCV genotyping are in use for type-specific amplification. The first method was introduced by Ohno *et al.* [26] in 1997 while the second method was developed by Idrees [27] in 2008. Comparatively, the method of Idrees (2008) [27] is more specific for Pakistani HCV isolates as it was developed in the light of local sequence data. However, most studies (nine articles) [3,9,10,13,14,16,20–22] used the method of Ohno *et al.* [26] while relatively less studies (five articles) [5,11,18,19,23] applied the method of Idrees. One study [24] utilized the method of Ohno *et al.* [28] while another one [12] used the method of Idrees [29]. In two studies [15,17] genotyping assay was not mentioned. Only a single study performed [8] sequencing for genotype authentication.

3.2. Genotypes and subtypes proportion

After proper analysis, genotype 3 (58.27%) was identified as the prominent HCV genotype in KP (Table 1) followed by genotypes 2 (12.39%), 1 (9.54%) and 4 (0.86%). Genotypes 5 and 6 were reported in rare frequency and constituted shares of 0.09% and 0.22% respectively. Two studies recorded the existence of genotype 5 and 6, indicating their rare presence [5,24]. Mixed genotypes contributed cumulative share of 7.17% to the total distribution pool. Among HCV subtypes, 3a accounted for 48.67% (Figure 1), followed by subtype 2a (10.91%), 3b (9.43%), 1a (5.84%), 1b (3.66%), 2b (1.45%) and genotype 4 with its undefined subtypes occupied a portion of 0.86%. Subtypes 1c, 2c, 3c, 5a and 6a were noted in seldom fashion revealing a combined contribution of less than 1%. In approximately 11.51% cases, the subtype could not be determined by the assay used, making those cases diagnostically untypeable. The percentage of untypeable cases was greater than all typeable subtypes except 3a (Figure 1).

3.3. Gender wise subtypes distribution

In Pakistan, HCV prevalence has been recorded equally among males and females in a former countrywide study [30]. We could identify only six studies [9,11,15–17,22] where gender information was available for the reported subtypes. All HCV subtypes were slightly greater among males as compared to females (Figure 2) except subtype 2b which depicted marginally greater proportion among females (f/m: 1.26). Subtypes 1c, 2c, 5a, 3c, 5a and 6a were not identified in the studies selected for gender wise subtypes comparison.

3.4. Studied groups category

Currently, fifteen studies [3,5,8,10–20,24] highlighted general population, one each revealed HCV subtypes in war-affected refugees [9] and health workers [21] whereas only two reports [22,23] were exclusively related to studies on risk groups. General population based studies and study on refugees unanimously demonstrated HCV 3a as the principal subtype. Among the risk groups, one study [22] was conducted on hemodialysis patients in which the leading HCV subtype was 3a. In the second study, intravenous drug users (IDUs) were highlighted [23], where 2a was the most pervasive HCV subtype. The presence of 2a in that study is an exception to all other reported studies in KP [3,5,8–22,24] which records 3a as the widespread subtype in HCV carriers.

Table 1
Details of hepatitis C genotypes and subtypes distribution studies conducted during 2005 till first half of 2016.

Reference	Proportion of different HCV genotypes and subtypes number (%)							
	1	2	3	4	5	6	Mixed	Untypeable
Idrees and Riazuddin (2008) [5]	1a = 54 (6.56) 1b = 21 (2.55)	2a = 63 (7.65) 2b = 7 (0.85) 2c = 1 (0.12)	3a = 397 (48.23) 3b = 157 (19.07) 3c = 7 (0.85)	19 (2.30)	5a = 1 (0.12)	6a = 3 (0.36)	42 (5.10)	51 (6.19)
Rauf *et al.* (2010) [9]	1a = 1 (4.00) 1b = 2 (8.00)	2a = 4 (16.00) 2b = 1 (4.00)	3a = 11 (44.00) 3b = 3 (12.00)	–	–	–	–	3 (12.00)
Safi *et al.* (2010) [10]	1a = 12 (10.10) 1b = 6 (5.00)	2a = 8 (6.70)	3a = 58 (48.70) 3b = 24 (20.20)	1 (0.80)	–	–	7 (5.90)	3 (2.50)
Ali *et al.* (2011) [11]	1a = 21 (6.00) 1b = 14 (4.00)	2a = 4 (1.31)	3a = 166 (86.91) 3b = 25 (13.08)	–	–	–	50 (16.39)	25 (8.19)
Inamullah *et al.* (2011) [12]	1a = 10 (5.40)	2a = 15 (8.10)	3a = 63 (34.10) 3b = 13 (7.02)	–	–	–	14 (7.60)	70 (37.80)
Ali *et al.* (2011) [13]	1a = 3 (1.50) 1b = 5 (2.50)	2a = 78 (39.00) 2b = 2 (1.00)	3a = 62 (31.00) 3b = 16 (8.0)	–	–	–	–	34 (17.00)
Afridi *et al.* (2013) [14]	1a = 10 (5.64)	–	3a = 160 (90.39) 3b = 1 (0.56)	1 (0.56)	–	–	5 (2.82)	–
Wasim *et al.* (2014) [15]	1a = 25 (11.30) 1b = 10 (4.50)	–	3a = 149 (68.10) 3b = 6 (2.70)	–	–	–	9 (4.00)	21 (9.50)
Ali *et al.* (2014) [16]	1a = 9 (7.43) 1b = 7 (5.78)	2a = 16 (13.22) 2b = 8 (6.61)	3a = 32 (26.44) 3b = 20 (16.50)	3 (2.47)	–	–	5 (4.13)	21 (17.35)
Ilyas *et al.* (2014) [17]	1a = 10 (5.00) 1b = 2 (1.00)	–	3a = 114 (57.00) 3b = 3 (1.50)	–	–	–	1 (0.50)	71 (35.50)
Waqas *et al.* (2015) [18]	1a = 39 (10.40) 1b = 11 (2.90)	–	3a = 227 (60.50) 3b = 25 (6.60)	–	–	–	20 (5.30)	53 (14.10)
Gul *et al.* (2016) [8]	1a = 14 (3.32) 1b = 49 (11.61)	2a = 21 (4.98) 2b = 16 (3.79)	3a = 192 (45.50) 3b = 22 (5.21)	–	–	–	97 (22.99)	11 (2.61)
Safi *et al.* (2012) [3]	1a = 14 (12.50) 1b = 10 (8.92)	2a = 11 (9.82) 2b = 2 (1.78)	3a = 48 (42.85) 3b = 25 (22.32)	–	–	–	–	2 (1.78)
Ali *et al.* (2010) [19]	1a = 3 (0.73) 1b = 3 (0.73)	–	3a = 240 (80.26) 3b = 25 (6.00)	–	–	–	28 (6.73)	116 (27.88)
Saleha *et al.* (2014) [20]	1a = 1 (1.00)	2a = 13 (12.00)	3a = 42 (38.00) 3b = 23 (21.00)	–	–	–	8 (7.00)	23 (21.00)
Khan *et al.* (2011) [21]	1a = 1 (4.34)	–	3a = 17 (73.91) 3b = 3 (13.04)	–	–	–	1 (4.34)	1 (4.34)
Khan *et al.* (2011) [22]	1a = 16 (14.28) 1b = 4 (3.57)	2a = 10 (8.92) 2b = 2 (1.78)	3a = 36 (32.14) 3b = 20 (17.85)	2 (1.78)	–	–	12 (10.70)	10 (8.92)
Ur-Rehman *et al.* (2011) [23]	1a = 3 (7.14)	2a = 17 (35.71)	3a = 14 (28.57)	7 (14.29)	–	–	–	7 (14.29)
Khan *et al.* (2014) [24]	1a = 19 (1.75) 1b = 22 (2.03) 1c = 2 (0.18)	2a = 235 (21.70) 2b = 28 (2.50)	3a = 179 (16.54) 3b = 17 (1.57)	6 (0.55)	5a = 3 (0.27)	6a = 7 (0.64)	23 (2.12)	–
Overall	433 (9.54)	562 (12.39)	2 642 (58.27)	39 (0.86)	4 (0.09)	10 (0.22)	322 (7.10)	522 (11.51)

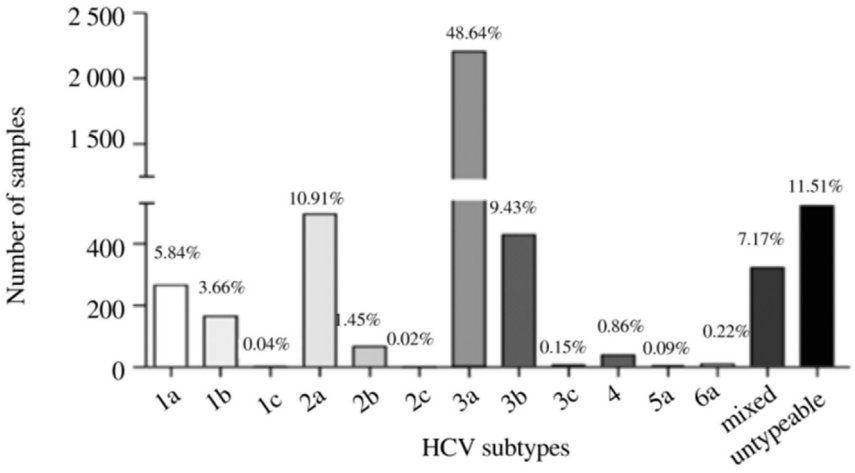

Figure 1. Overall proportion of various HCV subtypes.

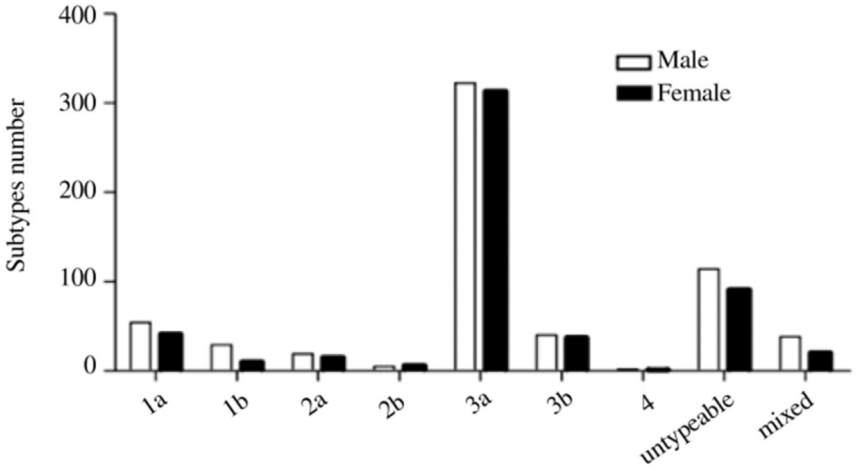

Figure 2. Gender-wise distribution of HCV subtypes.

3.5. Division wise genotypes distribution

The province of KP has been classified into seven administrative divisions previously. Among the total nineteen studies, only twelve studies [8–12,14–16,18,20–22] contained clear information about their respective geographical origin. Being urban regions, Peshawar [8,10,21,22] and Mardan [14–16,18] remained in limelight, reporting most genotype distribution studies. Limited studies (four articles) [9,11,12,20] concentrated other divisions including two studies from Malakand [9,12] and one each from Hazara [11] and Bannu [20]. No study was performed in Kohat and Dera Ismail Khan (D. I. Khan) divisions. The division wise subtype distribution pattern varied slightly; however, 3a was unanimously found to be the leading HCV subtype across all divisions (Figure 3).

3.6. HCV genotypes and hepatic steatosis

Hepatic steatosis is a known feature of prolonged chronic HCV infection [31]. Different HCV genotypes have different hepatic steatosis rate [31]. In this review, a single study was identified to assess the relationship of HCV genotypes with hepatic steatosis [10]. According to the study outcomes, genotype 3 was significantly associated with hepatic steatosis in infected individuals. In 77% individuals, steatosis was attributed to subtype 3a only.

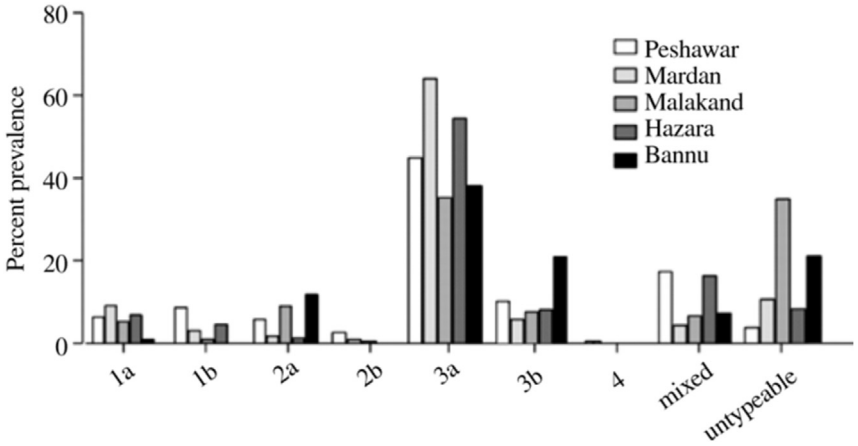

Figure 3. Distribution of HCV subtypes on the basis of geographical regions (KP divisions).

Table 2
Trend of HCV subtypes distribution on the basis of time (years).

Year	HCV subtypes percent prevalence													
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4	5a	6a	Mixed	Untypeable
2008	6.56	2.55	0.00	7.65	0.85	0.12	48.23	19.07	0.85	2.30	0.12	0.36	5.10	6.19
2010	2.86	1.96	0.00	2.14	0.17	0.00	55.27	9.30	0.00	0.17	0.00	0.00	6.26	21.82
2011	6.18	2.63	0.00	14.20	0.45	0.00	41.00	8.82	0.00	1.03	0.00	0.00	8.82	16.83
2012	12.50	8.92	0.00	9.82	1.78	0.00	42.85	22.32	0.00	0.00	0.00	0.00	0.00	1.78
2013	5.64	0.00	0.00	0.00	0.00	0.00	90.39	0.56	0.00	0.56	0.00	0.00	2.82	0.00
2014	5.36	3.43	0.16	22.12	3.01	0.00	43.25	5.78	0.00	0.75	0.25	0.58	3.85	11.39
2015	10.40	2.93	0.00	0.00	0.00	0.00	60.53	6.66	0.00	0.00	0.00	0.00	5.33	14.13
2016	3.31	11.61	0.00	4.97	3.79	0.00	45.49	5.21	0.00	0.00	0.00	0.00	22.98	2.60

3.7. Year wise HCV genotypes distribution

Starting from 2008 till the first half of 2016, different HCV subtypes exhibited random fluctuations with regard to their existence (Table 2). Most subtypes did not reveal any fixed pattern of escalation or decline during the mentioned period. The only HCV subtype that maintained a fixed abundant status over the years was found to be subtype 3a.

4. Discussion

In Pakistan, hepatitis C sustain endemic status with average adult anti-HCV frequency (6.7%) ahead of regional countries such as Afghanistan (1.1%), India (0.8%) and Bangladesh (1.3%) [2]. A recent review study reported in 2017, records a rise in the HCV seroprevalence in the country, revealing an overall anti-HCV frequency of 8.64% [32]. Escalated poverty, unchecked drug traffic from Afghanistan and the blatant use of septic procedures in the former years [7] and lack of substantial improvement in these practices till date [32] contributes toward the massive burden of the infection. According to the latest report [32], anti-HCV prevalence in KP is estimated to be 6.07%, indicating a huge burden of HCV like rest of the country. Information regarding HCV genotypes is vital in reckoning therapy duration, response to antiviral therapy and HCV-induced liver fibrosis progression pace [33,34].

In the current review, HCV genotype 3 and subtype 3a were identified to be the predominant types and subtypes respectively. The predominance of genotype 3 and subtype 3a was reported in all individual studies except the one conducted on IDUs [23], where proportion of 2a was ahead of all other subtypes. The pervasive nature of subtype 3a has been reported across the country [30]. Therefore, the pattern of HCV genotypes in KP indicates a distribution pattern in line with other parts of Pakistan. However, recently published study from the province point towards a possible rise in the proportion of previously uncommon HCV subtypes, whereas 3a still maintaining the dominant subtype status [8]. Generally, genotype 3 is abundant in the whole South Asian region [2]. In terms of treatment, genotypes 3 and 2 are comparatively sensitive to HCV treatment regimen of interferon [33]. Nevertheless, subtype 3a has been associated with high fibrosis and steatosis frequency [10,31,34]. Sustaining a remarkable proportion of about 48.67% in the current review, the association of 3a with greater fibrosis and steatosis tendency is an aspect of worry for HCV carriers in the region. As KP shares a porous border with Afghanistan where HCV subtype 3a is prevalent in IDUs [35], the spread of 3a in KP may be influenced by the unchecked influx of drugs including intravenous drugs [36]. Previously, the origin of subtype 3a in Pakistan has been traced back to areas of India and China called "Golden Crescent" region (renowned area for largest drugs production and supply) where intravenous drug use facilitated the transmission to Pakistan [37,38]. However, in KP precisely, 2a attains the status of most pervasive subtype in IDUs [23]. This point reveals gaps in our current knowledge regarding identification of the plausible HCV transmission origin in IDUs of KP. A phylogenetic analysis focusing IDUs is therefore imperative to unveil the mystery. Interestingly, the current review indicates a considerable number (11.51%) of untypeable HCV subtypes. The issue of untypeable subtypes manifests complications both diagnostically and therapeutically [13]. As revealed from HCV polymerase kinetic measurements, the estimated mutation rate of the virus is 1×10^{-3} per nucleotide per replication [39], which contributes toward fast evolution and genetic diversity. There is a high probability for these mutations to occur in diagnostically important sites of the genome [40] causing mistyping and emergence of untypeable cases. In developed countries, sequencing is performed in routine for the determination of underlying subtype in untypeable cases. However, in KP, the practice of sequencing is rare and the treatment duration is ascertained in term of HCV viral titer (qualitative and quantitative detection) [13], which is not a standard way of therapy duration estimation. Consequently, misdiagnosis and mistreatment are contributing toward non-responsiveness to the treatment regimen of interferon [1]. Further, presence of considerable untypeable subtypes need an updated genotyping assay devised on the basis of indigenous viral sequence data and implementation of sequencing strategies in the case of untypeable cases for determination of undetectable subtype.

Gender wise, HCV subtypes occurrence was comparatively slightly higher in males; nevertheless, this finding was statistically non-significant ($P = 0.836$). Other parts of the country reports equivalent HCV subtypes distribution in both genders [30]. Studies focusing HCV risk groups [22,23] were available scarcely (only two). As Pakistan is an underdeveloped country where limited awareness exists regarding HCV risk factors and standard aseptic procedures [41]. Therefore, further dedicated studies would be required to highlight the status of different risk groups clearly. In addition, the geographical location of various studies also highlighted the ubiquitous distribution of subtype 3a, making it the leading HCV subtype of the province. As majority of studies included in the current review have been conducted in mainstream areas and urban settings, there is a need of attention toward neglected southern divisions of KP including Kohat and D. I Khan, where no plausible genotyping studies have been carried out during the period under consideration.

Broadly, HCV has remained a major issue for this region overtime. Gradually, control measures and treatment options against HCV subtypes infection are improving in the developed world. Among the breakthrough drug discoveries, direct acting antivirals [42] are a ray of hope for the chronically infected individuals and expecting women (contraindication of ribavirin in pregnancy for possible teratogenic effects [43]) due to their striking success rate [42]. Unfortunately, developing countries are still deprived from access to direct acting antivirals. Health associated organizations and big pharmaceutical firms should ensure the availability of direct acting antivirals to developing countries on subsidized rate to tackle the burden of HCV in long run.

Conclusively, HCV 3a persists to be the predominant subtype across the province of KP. The enormous number of untypeable subtypes in most reported studies emphasizes the development of an improved genotyping system based on the indigenous sequence data. In addition, studies on determination of subtypes transmission pattern are indispensable for identification of transmission origin and implementation of effective control measures. Lastly, it is need of time to focus HCV risk groups and the neglected southern side of KP for estimation of more precise HCV genotype distribution pattern in the province.

Conflict of interest statement

The authors declare that they have no competing interest.

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