Pakistan J. Zool., pp 1-10, 2021. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20200107110132

Prevalence, Molecular Epidemiology and Drug Resistance Pattern of Human Immunodeficiency Virus Type 1 among Injecting Drug Users in Lahore, Pakistan

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Article Information
Received 03 January 2020
Revised 24 January 2020
Accepted 30 January 2020
Available online 29 June 2020

Authors' Contribution
SY and MFS collected samples. SY performed practical work. TY and NM conceived and designed the study and also supervised the research work. SY and AuR analyzed the data and wrote the manuscript. MZS, AN and ZT critically reviewed the manuscript.

Key words
HIV-1, Prevalence, Molecular epidemiology, Circulating subtypes, Drug resistance, Injecting drug users

ABSTRACT

Human immunodeficiency virus 1 (HIV-1) is common among injecting drug users (IDUs), and HIV-1 positive IDUs may have potential of HIV transmission among general population through different ways. Therefore, an understanding towards current status of HIV prevalence and genomic characteristics of circulating strains is crucial to devise and implement necessary interventions to control disease in developing countries like Pakistan. A total of 201 plasma samples were collected from IDUs. Samples were first screened using HIV-1 Ag/Ab Combo test and then antigen positive samples were amplified for HIV-1 pol gene (1084 bp) and analyzed. Initial screening showed a total of 49 HIV samples positive (24.37%; 95% CI: 18.96-30.76). A substantial association of HIV incidence was observed in individuals with HCV infection (36.84%; 95% CI: 28.55-45.99; $p<0.0001$) followed by individuals involved in practices of shared injection equipment (21.17%; 95% CI: 25.07-40.21; $p<0.0001$), injected previously used syringes (30.2%; 95% CI: 23.4-37.99; $p=0.0016$), sex with IDUs (37.78%; 95% CI: 25.11-52.37; $p=0.002$) and those with an age between 30-39 years (27.17%; 95% CI: 19.13-37.04; $p=0.039$). The *pol* gene-based phylogeny and subtyping classification categorized the under-study sequences representing subtype A (n = 12; 46.15%), CRF02_AG (n = 6; 23.08%), subtype C (n= 5; 19.23%) and subtype G (n = 3; 11.54%). Subsequent to genotyping resistance interpretation algorithm, one major (M46L) and two accessory (N88D, L89V) PIs mutations in the protease region while four NRTI (D67T, K70R/Q, M184V and T215F) and four NNRTI (V108T, E138A, V179I and Y181C) mutations in the reverse transcriptase region were observed. The present study concludes circulation of multiple subtypes of HIV-1 among IDUs and a continuous disease surveillance coupled with delineation of disease risk factors may provide a crucial insight into HIV prevention and treatment which could substantially curtail HIV epidemics in IDUs.

INTRODUCTION

Injection drug use has a potential influence on human immunodeficiency virus (HIV) transmission and has raised numerous HIV epidemics worldwide (Conrad *et al*., 2015; Mirzoyan *et al*., 2013; Paraskevis *et al*., 2011; Strathdee *et al*., 2010). According to world health organization (WHO), out of an estimated 15 million injecting drug users (IDUs), > 3 million (20%) are those living with active HIV infection around the globe. Injecting drug users account for approximately 10% of HIV infections counting up to 15.5% in East and Southern Africa and 30% in other countries. HIV infection was reported in 120 (81%) of the 148 countries in which 20-40% HIV prevalence was associated with injecting drug users in most of the Asian countries (Mathers *et al*., 2008). As Asian country, Pakistan is an HIV epidemic country and was considered to be a low prevalence country however in the view of recent increase in concentrated epidemics of acquired immune deficiency syndrome (AIDS) with

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0030-9923/2021/0001-0001 $ 9.00/0

passage of time; it is now in the group of "countries in transition" (Yousaf *et al*., 2011). Since 1987, the first HIV case in Pakistan (Khanani *et al*., 1988), the number of HIV cases has been steadily increased among population engaging in high risk practices (Yousaf *et al*., 2011). These risk factors, particularly the practice of injecting drug use put the Pakistani population at a high risk in spread of HIV infection, especially among injecting drug users (IDUs) (Samo *et al*., 2013).

HIV is an RNA virus with 9.2 kb size genome size. It is classified in the genus *Lentivirus* within the family *Retroviridae* (King *et al*., 2018). HIV-1 poses wide genetic variability which is represented by four major groups (M, N, O, and P) and of these M group is further classified into nine subtypes (A, B, C, D, F, G, H, J, and K) and many circulating recombinant forms (CRFs), highlighting undergo an evolution of HIV-1 in the population (Tongo *et al*., 2016). Currently, there are > 98 CRFs around the globe and in Asian countries HIV-1 subtypes A, B, C, D and G with multiple CRFs are circulating widely (Sarker *et al*., 2008). Such high genetic diversity reflects complexity of molecular epidemiology of virus within population. Characteristics of HIV-1 variants have an impact on infection transmission, diagnostic aspects, antiretroviral drug efficiency and vaccine development efforts (Kline *et al*., 2019; Zanini *et al*., 2015).

HIV genome contains several genes from which *pol* codes for the viral enzymes reverse transcriptase (RT), integrase (IN) and protease (PR); functionally important for virus replication (Fernandes *et al*., 2016) and occurrence of substitutions in these regions of *pol* protein correspond to subtype differentiation and antiretroviral drug resistance (Nagata *et al*., 2017; Abram *et al*., 2010). The survival fitness of these emerging variants is attributed to mutations in its genome for host adaptation that enable them to evade immune system of the host. Such mutations may have much influence in drug resistance pattern and may cause halts in antiretroviral drug therapy (ART) for the treatment of HIV infection (Little *et al*., 2002; Wallis *et al*., 2010). Therefore, it is always problematic to develop an efficient prophylactic intervention using same class of drugs. Along with occurrence of mutations, the genomic variability of HIV in terms of distribution of different subtypes and CRFs raised concerns about drug efficacy and enforcing to insight the susceptibility pattern of those drugs being administrated in population in which various HIV-1 subtypes and CRFs are circulating.

Owing the circulation of different HIV-1 subtypes and CRFs, several studies have highlighted increasing number of HIV-1 variants among infected individuals (da Guarda Reis *et al*., 2017). In the past, several studies have been conducted to estimate the HIV-1 prevalence in the general population of Pakistan, however; information related molecular epidemiology of currently prevalent subtypes and CRFs among IDUs is scarce. Although HIV-1 prevalence in IDUs has been investigated in most of cities of different provinces, including Sindh, KPK and Baluchistan, and very few cities of Punjab province, but to best of author's knowledge this first ever study to estimate the prevalence of HIV-1 and its genotyping in IDUs in Lahore. Therefore, the aim of the current study was to comprehensively estimate the prevalence of HIV-1, molecular epidemiology of HIV-1 subtypes and CRFs and drug resistance pattern in IDUs of Lahore because of the high ratio of usage of injecting drugs and more probable to be an occurrence of HIV-1 prevalence in this population. This information would be helpful to better understand the HIV-1 epidemiology and drug resistance pattern in IDUs and could be associated with genetic evolution and foundation in anticipating the scope and effectiveness of ART in IDUs.

MATERIALS AND METHODS

Ethical statement

This study was carried out in accordance with the recommendations of ethical committee and all essential procedures for sampling were taken after approval by Institutional Review Committee of Institute of Public Health, Lahore vide letter # IRC: 41/17.

Sample collection and HIV-1 detection

Under this cross sectional study, 201 plasma samples were collected from people who inject drug (PWID) from different location of Lahore city, Pakistan during February 2017- October 2017. All subjects were male. All participants supplied written informed consent for specimen collection and subsequent analyses. A questionnaire was designed to record of demographic information such as age, marital status, level of education and history of injecting drug use practice. Samples were initially screened using HIV antigen/antibody Combo test (Alere. Determine TM HIV US) according to manufacturer's instruction and interpreted. The screening test had potential to detect both antibodies and antigen simultaneously. In this test antigen is exclusive to HIV type 1, while the antibodies may correspond to either HIV-1 or HIV-2. Besides, HCV surface antigens test was also conducted using RAPID ICT test kit (One-step Hepatitis C Virus Test, Alere, Cat. No 02FK10) was used according to manufacturer's instructions. After initial screening, from HIV-1 antigen positive samples, the viral RNA was extracted using QIAamp DSP RNA Mini Kit (Qiagen, Germany) according to manufacturer's instructions. The *pol* gene (1084 bp) of HIV-1 was amplified using one-step reverse transcriptase

polymerase chain reaction (RT-PCR), High-Fidelity Taq polymerase SuperScript III (Life Technologies, USA) in the reverse transcriptase (RT)-nested polymerase chain reaction according to previously described protocol (Zhou *et al*., 2011). Amplified PCR products were further purified and sequenced by Sanger sequencing method using both reverse and forward primers (Applied Biosystems Prism 310 Genetic Analyzer).

Subtyping classification and phylogenetic analysis

The under-study sequences were examined for subtype determination using REGA HIV-1 Subtyping Tool (Version 3.0). Moreover, subtyping of under study sequences was further confirmed using jumping profile Hidden Markov Model (jpHMM) available online. Afterward, the obtained sequences were aligned with the reference sequences using ClustalW method in BioEdit software (Hall, 1999). Phylogenetic tree was constructed using neighbor-joining method and Kimura-two parameter model with general time reversible, gamma distribution and invariants nucleotide sites calculated on 1000 bootstraps replication in MEGA 6.0 software (Tamura *et al*., 2013). All of the mplified sequences from the study were submitted in the GenBank and accessible with accession numbers MN410946-MN410971.

Estimation of antiretroviral drug resistance

Initially, the quality of sequences was assessed using Calibrated Population Resistance (CPR) tool version 6.0 (http://cpr.stanford.edu/cpr.cgi) and removed those sequences that did not pass the quality criteria. To investigate the presence of transmitted drug resistance mutations (TDRMs), genotyping resistance interpretation algorithm was employed using protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI) and nucleoside reverse transcriptase inhibitor (NRTI) drugs available in Stanford HIV database.

Statistical analysis

To investigate possible association between categorical variables (demographic particulates) and HIV-1 positivity or negativity IBM SPSS statistics (version 21.0) was used. A level of $p < 0.05$ was considered as statistically significant.

RESULTS

HIV prevalence

Alere HIV Combo test showed detection of 49 HIV positive samples (24.37%; 95% CI: 18.96-30.76). These included either detection of antigen alone (n = 26, 50.03%; 95% CI: 39.38-53.06), and for both antigen and antibodies (n = 23, 46.94%; 95% CI: 33.7-60.62). Cumulatively, a significant association of HIV occurrence was observed in individuals having HCV infection (36.84%; 95% CI: 28.55-45.99; p<0.0001) followed by individuals involved in practices of shared injection equipment (21.17%; 95% CI: 25.07-40.21; p<0.0001), used syringes (30.2%; 95% CI: 23.4-37.99; p=0.0016), sex with PWID (37.78%; 95% CI: 25.11-52.37; p=0.002) and those with an age between 30-39 years (27.17%; 95% CI: 19.13-37.04; p=0.039). Between other univariates the association was non-significant such as single/divorced subjects (28.12%; 95% CI: 19.91-41.77, p=0.187), subjects with a no formal education (26.92%; 95% CI: 20.04-35.13, p = 0.256) and those lived as street dwellers/beggars (25.58%; 95% CI: 18.83-33.74, p = 0.596). The Cramer's V statistical test revealed a strong and very desirable association of HIV occurrence in individuals with HCV (0.33), moderate strong and desirable association of HIV occurrence in individuals having shared syringe equipment practice (0.285), moderate and acceptable association of HIV occurrence in individuals between 30-39 years of age (0.225), having practice of used syringe (0.22) and having sex with PWID (0.201), very weak and not generally acceptable association of HIV occurrence was observed in individuals lived single (0.093) and individuals with lack of formal education (0.08), whereas, no association of HIV occurrence was observed in individuals were homeless and lived as street dwellers/beggars (0.038) (Table I).

Subtyping and phylogenetic analysis

Of these 49 Alere HIV Combo test-HIV positive samples, 26 antigen positive samples were amplified in both first and second round of RT-PCR. Subsequently, substantial analysis of genotyping, phylogenetic and drug resistance analysis was done. Essential analysis for subtyping classification categorized the under-study HIV-1 sequences into three HIV-1 subtypes (A, C and G) and a circulating recombinant form (02_AG). Cumulatively, subtype A was found to be predominant (46.15%) followed by 02_AG CRFs (23.08%), subtype C (19.23%) and subtype G (11.54%). The *pol* gene-based phylogenetic analysis clustered study sequences into four distinct clades representing subtype A (n = 12), C (n = 5), G (n = 3) and 02_AG CRFs (n = 6). Sequences representing subtype A were clustered with HIV sequences originating from Pakistan (JN620529, JN620500 and JN620510) and Uganda (AY803472), 02_AG CRFs sequences were clustered with African originated sequence (AY371134), subtype C sequences were clustered with Indian (AF067155) and Japanese (AB023804) originated sequences and subtype G sequences were clustered with HIV sequence originating from Singapore (EU715229) (Fig. 1).

Table I. Demographic characterization and risk factor analysis of HIV-1 infection among injecting drug users.

Risk Factors	Total no.	Positive	Percentage	95% CI	Odd ratio	Risk ratio	p value	Cramer's V-value
18-29	68	15	22.06	13.85-33.26	0.423	0.581	0.039*	0.225[c]
30-39	92	25	27.17	19.13-37.04				
>40	41	9	21.95	12-36.7				
Married	137	29	22.63	15.16-28.75	0.636	0.713	0.187[NS]	0.093[d]
Single/Divorced	64	19	28.12	19.91-41.77				
Some formal education	71	14	19.72	12.13-30.42	0.667	0.732	0.256[NS]	0.08[d]
Illiterate	130	35	26.92	20.04-35.13				
Yes	143	46	32.17	25.07-40.21	8.694	6.219	<0.0001***	0.285[b]
No	58	3	5.2	1.87-14.85				
New	52	4	7.7	3.03-18.17	0.202	0.263	0.0016**	0.22[c]
Used	149	45	30.2	23.4-37.99				
Yes	45	17	37.78	25.11-52.37	0.401	0.538	0.02*	0.201[c]
No	63	9	9.52	7.71-24.98				
Don't know	93	23	24.73	17.08-34.38				
Yes	114	42	36.84	28.55-45.99	6.667	4.578	<0.0001***	0.33[a]
No	87	7	8.04	3.95-15.69				
Homeless/street dwellers/beggars	129	33	25.58	18.83-33.74	1.203	1.151	0.596[NS]	0.038[e]
Jobless	72	16	22.22	14.17-33.09				
Grand total	201	49	24.37	18.96-30.76				

Note: *, significant (p<0.05); **, high significant (p<0.01); ***, very high significant (p<0.001); NS, non-significant (p>0.05); a, strong and very desirable association; b, moderately strong and desirable association; c, moderate acceptable association; d, very weak and not generally acceptable association; e, no association

Table II. Major and minor mutations in protease (PR) gene and reverse transcriptase (RT) gene of under-study HIV-1 sequences.

HIV-1 sequences	Subtypes	Protease gene		Reverse transcriptase (RT) gene	
		PIs major	PIs accessory	Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
PK/LHR/IDU/05 MN410946	C	None	None	T215F	None
PK/LHR/IDU/119 MN410947	C	None	None	T215F	None
PK/LHR/IDU/13 MN410955	A	None	None	T215F	E138A
PK/LHR/IDU/122 MN410956	A	None	None	None	E138A
PK/LHR/IDU/156 MN410950	C	M46L	None	None	None
PK/LHR/IDU/23 MN410951	02_AG	None	L89V	None	None
PK/LHR/IDU/139 MN410968	02_AG	None	L89V	T215F	None
PK/LHR/IDU/46 MN410952	G	None	None	None	V179I
PK/LHR/IDU/22 MN410953	G	None	None	T215F	V179I
PK/LHR/IDU/172 MN410949	C	M46L	None	None	None
PK/LHR/IDU/49 MN410954	G	None	None	D67T, K70R, M184V	V108I, Y181C
PK/LHR/IDU/140 MN410962	A	None	N88D	None	None
PK/LHR/IDU/176 MN410964	A	None	None	T215F, K70Q	None
PK/LHR/IDU/56 MN410965	A	None	None	T215F	E138A
PK/LHR/IDU/15 MN410970	02_AG	None	None	T215F	None

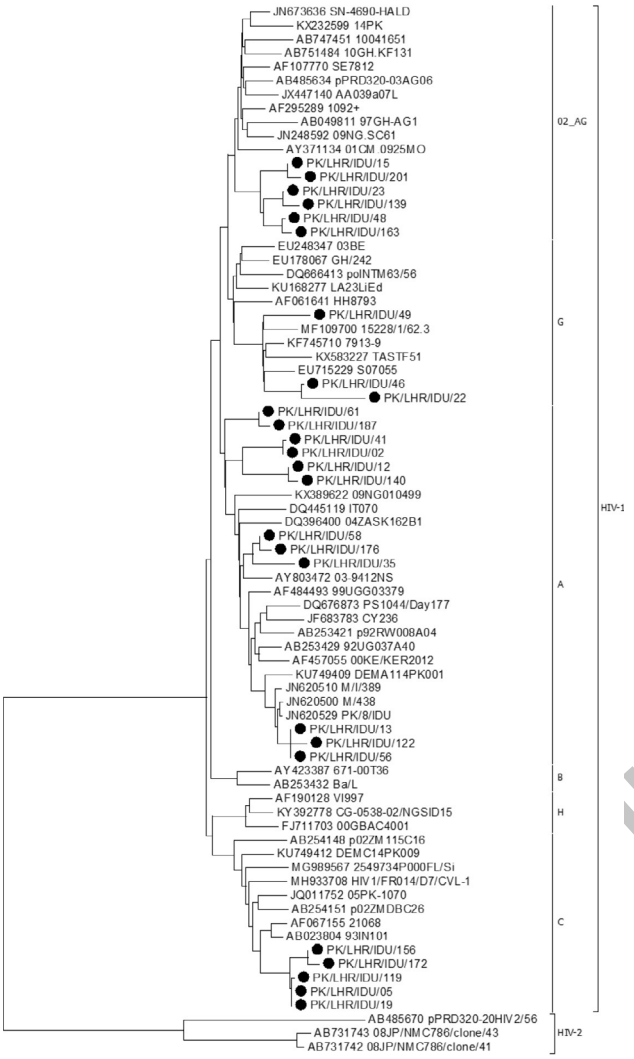

Fig. 1. Based on *pol* gene, the phylogenetic analysis was constructed using studied HIVs and previously characterized strains representing different subtypes originating from the globe. The phylogeny tree was constructed using neighbor-joining method in MEGA version 6 software. The under-study HIV strains are highlighted as black circle.

Antiretroviral resistance pattern

Cumulatively, the drug resistance analysis showed one major (M46L) and two accessory (N88D, L89V) PI mutations in protease region while four major NRTI mutations (D67T, K70R/Q, M184V and T215F) and four NNRTI (V108T, E138A, V179I and Y181C) were observed in reverse transcriptase region of *pol* gene. Among PIs, TPV/r showed the highest susceptibility level (100%) while NFV (19.23%) presented highest resistance level as compared to ATV/r (11.54%), SQV/r (11.54%) and FPV/r (7.69%) (Table II). Within NRTIs class, ABC showed the highest susceptibility level (100%) followed by 3TC, AZT, D4T, DDI, FTC (92.3%), whereas, a highest resistance level was observed against TDF (30.76%). Within NNRTIs class, NVP showed highest susceptibility level (100%) followed by DOR and EFV (92.3%) while highest resistance level was observed against ETR and RPV (15.38%) (Table II).

DISCUSSION

The current study revealed a high risk of HIV occurrence in the individuals involved in practice of shared injection equipment and injected previously used syringe because injection drug use is a potential source of HIV transmission among IDUs (Degenhardt *et al*., 2017; Stone *et al*., 2018). This is because, HIV may remain viable in the contaminated syringe for several weeks which has become a potential source of HIV transmission (Kaplan and Heimer, 1992). Most studies have revealed that the occurrence of HIV in IDUs is likely to be linked to practices of injecting with previously used syringe and/or borrowed from another PWID. The transmission of HIV through sharing syringes is a proficient route of infection transmitting (Ball *et al*., 2019; Conrad *et al*., 2015; Stone *et al*., 2018). There have been many examples of very rapid spread of the HIV-1 via shared syringe practices among PWIDs in resource rich and poor settings (Des Jarlais *et al*., 2016; Rhodes *et al*., 2002). Overall, 24.37% HIV prevalence was observed herein in IDUs in Lahore city which is slightly higher than other cities observed in previous studies such as 23% in Karachi, 19.6% in Sukkur and 18.3% in Hyderabad (Altaf *et al*., 2009; Bokhari *et al*., 2007). The practice of injecting drug use is considered serious risk factor that could put the country in danger of facing a rapid spread and increase of HIV-1 infection. Although Pakistan is a low HIV prevalence country, however, the IDUs are playing a vital role in HIV transmission (Strathdee *et al*., 2010). Since the first HIV epidemic among PWIDs in Larkana (Shah *et al*., 2004), HIV-1 has now become well established in PWIDs across the country and despite of various precautionary efforts, the epidemics have relatively expanded among PWIDs with the passage of time (Ali *et al*., 2017). A strong association was also observed in PWID involved in sexual activities because after sharing needles the major route of HIV transmission is sexual transmission for possible spread of disease among population of large geography (Ball *et al*., 2019; Conrad *et al*., 2015; Stone *et al*., 2018). The tendency for rapid transmission of HIV-1 between those IDUs involved in sexual activities has become a hallmark of epidemics in Pakistan (Strathdee *et al*., 2010).

In the current study, a high HIV prevalence was

observed in PWIDs aged 30-39 years which are in agreement of previous study where high HIV prevalence was observed in middle aged individuals (Kooij *et al.*, 2016). Overall, an estimated 5 million young PWID aged 25–35 years were living with HIV and accounted for new HIV infections globally (Feng *et al.*, 2014; Yu *et al.*, 2016). The comparable trend was also found in a previous study where rate of HIV incidence was significantly higher in middle-aged PWIDs (30-40 years) than other age groups (Hakre *et al.*, 2015). The possible reason could be that middle-age individuals are likely to be involved in active sexual life and drugs use practices as compared to young and old PWIDs. Furthermore, a strong association of HIV occurrence was observed in those PWIDs with co-infection of HCV. This is not intersecting because sharing of equipment used for IDUs causes substantial disease burden and such practices of sharing needles and injecting previously used syringe are also a main source of HCV transmission and epidemics not only in PWIDs but also among general population (Eckhardt *et al.*, 2017; Tseng *et al.*, 2007).

Besides the estimation of HIV-1 prevalence, genetic diversity of HIV-1 revealed the circulation of three subtypes (A, G, C) and a CRFs (02_AG) among IDUs. To best of our knowledge, this is the first ever study to explore different HIV-1 subtypes and CRFs circulating in IDUs in Lahore. HIV-1 Subtyping is crucial to understand genetic evolution of circulating HIV-1 isolates in the world (Pond *et al.*, 2009) and, *pol* gene of HIV-1 is an important marker to accurately delineate HIV-1 genetic diversity (Cantão *et al.*, 2018; Gonzales *et al.*, 2001; Hernandez-Sanchez *et al.*, 2018; Song *et al.*, 2018). The identification of different HIV-1 subtypes and recombinant form within specific population within same region highlights its propensity to be a highly mutable virus, therefore, time to time molecular epidemiological studies are required to conduct at a much higher resolution in future. Previously irrational use of syringes and frequent sexual contact with another either PWID or normal partner has previously been found associated with the spread of HIV-1 and emergence of subtypes (McAuley *et al.*, 2019). Similar evidences of shared syringes and injecting previously used syringes were also observed in current study.

In a previous study, practice of injecting previously used syringe was also anticipated to influence in emergence of novel HIV-1 subtypes or inter-subtypes and different circulating recombinant forms (Lin *et al.*, 2006; Sarker *et al.*, 2008). Although, findings of this study are in the agreement with the previous published study conducted in Sindh, Pakistan, wherein many HIV-1 subtypes and different inter-subtypes were reported among general population (Chen *et al.*, 2016; Khan *et al.*, 2018; Khanani *et al.*, 2011). However, there is a paucity of information on circulation of subtypes and recombinants forms of HIV-1 in IDUs. Consistence to Los Alamos HIV database and previous studies, the current study revealed subtype A as abundant HIV-1 subtype circulating in IDUs. Such variety of recombinant form (02_AG) evolving from two pure parental subtypes (A and G) indicates emergence of transmissible and viable variants, enhancing genetic diversity of HIV-1.

The possible explanation of emergence of CRF02_AG (recombinant form) could be due to co-circulation of multiple subtypes (A and G) in same region (Lahore) and/or co-infection with multiple subtypes of HIV-1 (Leal *et al.*, 2008; Thomson *et al.*, 2002). It is well-known that there is likely to be a possibility of the emergence of HIV-1 CRFs in specific region where multiple subtypes are circulating at a time (Chen *et al.*, 2012). Noteworthy, the high abundance (n = 6) of HIV-1 recombinant form (02_AG) was observed here in rather than the parental subtype G (n = 3). An in vivo investigation of HIV-1 recombination has revealed that an increase in the proportion of HIV-1 recombinant genomes may coincide with the loss of parental subtype (Iglesias-Sánchez and López-Galíndez, 2002). Indeed, an increase in the prevalence of CRFs may related to the vanishing of parental subtypes, which are usually found at the low frequency (Sanabani *et al.*, 2006). This could be reason of low frequency of subtype G prevailing in the field as compared to CRF02_AG. Consistently, in other Asian countries like Malaysia, the HIV-1 epidemic is experiencing a gradual replacement of original/initial predominant parental subtype by CRFs (Lau and Wong, 2013). As a consequence of HIV-1 recombination, the genetic variability may enhance the opportunity of host adaptation for various CRFs following modification in selection pressure. Therefore, these emerging CRFs and other variants may have ability to escape host immune responses (Streeck *et al.*, 2008) and/or develop resistance to antiretroviral drugs (Bennett *et al.*, 2009).

Considering the resistance to antiretroviral drugs, all under-study sequences were associated to relatively low level of resistance to PIs however low-high level of resistance to NRTIs and NNRTIs. Herein, the evidences of only one major (M46L) and one accessory (L89V) mutations in sequences related to PIs suggests that the virus is susceptible to all protease inhibitors. Theses observed findings correspond to previous study reported from Sindh province Pakistan where one major resistance associated substitution was observed in study sequences (Khan *et al.*, 2018). Besides, a low-level to high-level drug resistance to TDF, ETR and RPV was also observed due to occurrence of few mutations against the potential sensitivity of NRTIs (T215F, D67T, K70R/Q, M184V) and NNRTIs (E138A,

V179I, V108I, Y181C). Such mutations have deleterious impact on the susceptibility of antiretroviral drug and may create enormous obstacles to AIDS first-line therapy around the globe (Girard *et al*., 2006). Another study claimed minor mutations with non-significant impact in RT region of HIV-1 *pol* gene (Gatanaga *et al*., 2010; Khan *et al*., 2016; Shah *et al*., 2011). Low level of drug resistance in infected population of Punjab is satisfactory and, combination of PIs with NRTIs and NNTRIs, could be continued to treat affected population. Therefore, more studies should be conducted to monitor the trends in mutations so that necessary interventions could be applied in terms of change in regimens immediately for effective treatment and subsequent control of infection in the future.

CONCLUSION

Current study concluded the extreme genetic heterogeneity of HIV-1 strains prevailing in IDUs and highlighted the significance of continuous monitoring the molecular epidemiology of HIV for the better control of epidemics. Findings of present study emphasize on more focused and intensive awareness programs for safe injection practices throughout the country, especially among IDUs. Local officials could possibly use subtype prevalence data coupled with the demographic details and other molecular analysis to identify on-going HIV-1 transmission and implementation of a tailored combination of interventions for the substantial effect on local HIV epidemic trajectories.

ACKNOWLEDGMENT

The current study was carried out under the project of Punjab AIDS Control Programs (PACP), Primary and Secondary Health Care Department, Government of Punjab, Pakistan. We would like to thank the team leader and staff members of Institute of Public Health (IPH) Lahore for their help in collecting surveillance information and providing necessary laboratory facilities.

Statement of conflict of interest

The authors have declared no conflict of interest.

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