



# Prevalence of HCV-HIV Co-Infection with Intravenous Drug Users in Central Punjab, Pakistan

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## ABSTRACT

Hepatitis C and acquired immunodeficiency syndrome (AIDS) are major viral infectious diseases around the world especially in developing nations with poor healthcare facilities. Owing to blood-borne transmission, sharing of needles by the Intravenous Drug Users (IDUs) aggravates the spread of Hepatitis C and AIDS. Human immunodeficiency virus (HIV), causative of AIDS, mainly leads to reduced immunity provoking increased vulnerability to opportunistic infections such as Hepatitis C virus (HCV). Here, we present co-occurrence of HCV and HIV in male IDUs from four major cities of Central Punjab, Pakistan. Initial screening for HIV and HCV was performed using ELISA and real-time PCR. A high HCV and HIV co-infection was revealed in Lahore (80.77%) followed by Faisalabad (81.93%), Sargodha (76%) and in Nankana (62%). A strikingly high level of co-infection was observed compared to single infection. These results suggest a high rise in HCV and HIV co-infection among the HIV positive IDUs. Estimate of the CD4 cell count showed a strong correlation of high HCV and HIV viral titre with low CD4 count. In co-infected individuals, a significantly decreased level of CD4 cells was seen in both medium high and very high viral load categories with a frequency of 19.23% and 76.92%, respectively. Taken together, current study highlights the increased prevalence of HCV co-infection in HIV infected IDUs with lower CD4 count. These results strongly support an immediate investigation of multiple viral infections routine IDUs to guide the eradication of these fatal viral diseases.

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AZ conceived, designed and wrote the research article. MAS proofread the study. HJ confirmed the data and sources. MAUK helped in writing of the article and analysis of the data.

## Key words

Intravenous drug users (IDUs), Hepatitis C virus, Human immunodeficiency virus, Hepatitis B Virus, HCV-HIV co-infection, CD<sub>4</sub> count

## INTRODUCTION

High rise in medically important viruses such as human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B virus (HBV) has seriously risked public health worldwide. During the past few years, local viral surveillance by AIDS controlling authorities in different parts of Pakistan have demonstrated endemic prevalence of HIV infections among intravenous drug users (IDUs) (Mansha *et al.*, 2017). The estimated burden of HIV in Pakistan remained relatively low; however, currently the increasing number of cases has brought the national health care authorities at an alarming situation in terms of public

health and safety standards. This has shifted the trend from low to high rise in HIV infection in the country. In 2019, more HIV cases were reported with a high mortality rate (Wahid, 2019) which was then followed by two major HIV outbreaks in the same year in Rotodero among infants and in Faisalabad, triggering health catastrophe in Pakistan (Zahra *et al.*, 2019; Mir *et al.*, 2020). Most of the HIV positive patients were drug addicts and had acquired HIV due to reuse of syringes and needles, unhealthy practices of local quacks and dental surgeries. Surprisingly, co-infection with HCV and HIV, and HBV and HIV occurs frequently worldwide especially in HIV infected IDUs. This is primarily attributed to the blood borne transmission of these viruses (Kiani *et al.*, 2021). This serves as a major contributing risk factor for both HIV and HCV infection especially in IDUs where needle sharing and contact of infected blood is the main culprit of the onset of the disease. It has been reported that HCV and HIV co-infection prevails in 90% of IDUs (Alter, 2006; Aceijas and Rhodes, 2007). In HCV and HIV co-infected patients, accelerated hepatic fibrosis and higher rate of liver degeneracy are same of the causes of mortality as compared to non-HIV

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infected patients. Recent findings have revealed various mechanisms such as increased hepatic apoptosis, raised levels of inflammation and microbial translocation from the gut which contribute to impairment of HCV immune response and rapid mortality in co-infected patients (Maier and Wu 2002; Andreoni *et al.*, 2012). High rates of HCV infection among HIV positive patients has been mainly reported in injecting drug users (98%), bisexuals (80%) and homosexuals (3-15%). Approximately, 2.3 million people are co-infected with HCV and HIV infection according to WHO reports, and the majority of this population (1.3 million) belongs to IDUs (Platt *et al.*, 2016). Approximately, 83,468 individuals are infected with HIV alone in Pakistan of which only 7568 are registered in different AIDS control centres across the country. Majority of these HIV positive people do not receive the recommended ART antiretroviral therapy thereby becoming one of the significant risks for the transmissions of viral infections among local communities (Imran *et al.*, 2016).

Despite many strict measures and checks by the narcotics law enforcement agencies, cannabis has been one of the most commonly used drugs in Pakistan among individuals aged 15 to 64 years (Yaqub, 2013). Multiple other drugs such as opiates, heroin and a variety of painkillers were also commonly used by individuals. Majority (~73%) of these drug users share a syringe (Yaqub, 2013). This situation is highly aggravated due to the country's widespread permeable borders shared with Afghanistan which is one of the largest opium producers (Strathdee *et al.*, 2003). However, the accessibility, approachability and high costs have reduced snorting and shooting of heroine directly, switching them to syringe sharing with other easily available drugs. These factors support the expanded use of shared syringes and the number of IDUs in the country (Vermund *et al.*, 2006). Sharing of syringe needles is implicated as a major cause of contracting HIV and hepatitis. In Pakistan, nine million people are drug users among which 10% represent the IDUs. As per 2005 reports, percentage of HIV in IDUs was about 6.2% in Pakistan (Waheed *et al.*, 2009; Bergstrom *et al.*, 2015). Lack of awareness about HIV patients and its mode of transmission, social and religious taboos, high risky behaviours of unsafe sex, commercial sex workers, homosexuals (males who have sex with males MSM) add up in the infected population activating the fast spread of HIV (Lane *et al.*, 1985). Ever since HIV has been recognized as a deadly agent for immunocompromised individuals, CD4 cell count has played a major role in prognosis of HIV for initiation of antiretroviral therapy (ART) and making diagnostic decisions easier for clinicians (Phillips *et al.*, 1992). Significance of CD4 cell

count attains major importance as it prescribes the exact status of a patient's immune system and possibilities of opportunistic infections with respect to diagnostic inference in individuals with HIV progression. If advanced stage of HIV infection remains untreated, the CD4 count is reduced to about  $200 \times 10^6/L$  of blood (Sabin *et al.*, 2000; Ledergerber *et al.*, 1999) and the viral load rises to about 10,000 copies/ml or more (Buavirat *et al.*, 2003). Immediate medication against viral infection provides reservation of the HIV specific CD4 cells and lowers the viral load which reduces the progression of infection and fatality. Previous studies have highlighted the relationship between baseline CD4 cell count and HIV load responses in HCV and HIV co-infected individuals (Buavirat *et al.*, 2003; Rhodes *et al.*, 2005).

In both HIV and HCV, viral transmission is primarily through blood borne which could be either by sexual contact or by sharing of needles in IDUs. Contaminated blood products, unchecked blood transfusions, reuse of needles for ear and nose piercing, unsterilized surgical and dental instruments, razor sharing by local barbers have instigated the spread and transmission of such infections over the time (Vickern *et al.*, 2009; Kuo *et al.*, 2006). Due to under sourced community, limited domestic infrastructure for housing, sanitation, and medical facilities, Pakistan remains assailable to such health calamities on the massive scale.

The current study was designed to analyse the frequency of HCV and HIV co-infection among IDUs in different cities of Pakistan, which may lead to liver failure and increased morbidity in IDUs. The data revealed an increasing incidence of HCV and HIV co-infection in the targeted population of IDUs from different parts of the country and highlighted the prevailing high risk of co-infection among compared to single infections. Additionally, observations on the correlation between baseline CD4 cells and viral load of HIV and HCV confirm early diagnosis and possible start of the antiretroviral therapy for better prognosis.

## MATERIALS AND METHODS

A descriptive cross-sectional study was conducted from January 2019 to May 2019 in collaboration with Nai Zindagi (a National NGO) and Punjab AIDS Control Programme (PACP). Samples were collected from three different centers of the organization at Lahore, Faisalabad and Sargodha in HIV screening camps. Samples of IDUs were also collected from Shah Kot Darbar Nankana region (near to Lahore) and were included in the study. All blood samples from study population were directed to Punjab AIDS Control Programme (PACP). The

study was approved by the Institutional Review Board of University of Central Punjab and PACP. Informed consent was taken from all the enrolled subjects. From all participants, three milliliters of blood was collected from male IDUs. Serum was separated and stored at 4°C until use. For HBV screening, the Determine™ HBsAg point-of-care test (Alere Inc., MA, USA) was applied whereas for HCV screening, a SD Bioline HCV (Standard Diagnostics Inc, Republic of Korea (02FK10) was used under the guidelines suggested by WHO for diagnosis of HIV in resource limited countries. The Alere™ HIV Combo Rapid Test of Alere Medical Co. Ltd., Matsudochi, Japan), Uni-Gold HIV of Uni-Gold, Trinity Biotech, Ireland; #1206502 and SD Bioline HIV-1/2 3.0 of Standard Diagnostics, Kyonggi-do, South Korea. HIV, HCV and HBV were also detected by Real time PCR. The HIV RNA in human plasma was amplified using the Cobas 4800 HIV-1, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1. The HCV RNA amplification was performed using Artus HCV RG RT-PCR kit. The HBV, RNA was isolated using QIAamp DSP Virus kit and amplification was performed using QIAgen Artus<sup>R</sup> HBV RG PCR kit on the Rotor G PCR system. The CD4 counting was conducted with PIMA CD4 cartridge and Alere PIMA™. Quantitative data was analysed and presented in percentage and frequencies. Pearson correlation tests were used to obtain the p-value. The differences between the groups were considered significant when 'p' value was <0.05. The scatter plot statistical analysis was performed using "R" programme. The regression line is a straight line demonstrating the best fit for measurement points on scatter plot.

## RESULTS AND DISCUSSION

A total of 308 confirmed IDUs individuals were recruited randomly from four different cities of Punjab. All participants were males with a median age up to 31 years  $\pm$  9.64 years. A total of 104 IDUs were located in Lahore. Among 14 individuals, a percentage frequency of 13.46% was noticed on real-time PCR for HIV single infection (i.e. HIV mono-infection), 5 (4.81%) were positive for HCV mono-infection, none of them were detected positive for HBV mono-infection and the HCV-HIV co-infected individuals represented a high percentage showing 84 (80.77%) (Table I). Similar results were observed when data of other cities was analysed. Sargodha demonstrated high percentage frequency of 16 (76%), Faisalabad 68 (81.93%) and Nankana 61.90% of the HCV and HIV co-infected individuals as compared to HIV, HCV, HBV mono-infection (Table I). For further validation, subjects were also tested for HBsAg by rapid testing method. HBsAg was reactive only in 3 individuals with a low frequency

of 0.32% and 0.65% (Table II). Here we categorized co-infection according to our observations in three different categories: HCV and HIV, HBV and HIV co-infection and HCV, HBV and HIV triple infection, respectively. The analysis of three different categories revealed 98.77% frequency of HCV and HIV co-infection (Table II) with a significant difference (Fig. 1).

Interestingly, only a fraction (1.30%) (Table I) of participants in the studied IDUs were devoid of screened infection. The ART (anti-retroviral therapy) is mainly recommended on the level of CD4 counts in conjunction with high viral loads that provide the baseline prognostic markers for HIV diagnosis. The viral loads are the measures of infectivity with low, high or very high ranges. This defines the progression of viral infection and transmission within an individual. Since the IDUs enrolled in the study were ART naïve at the time of registration, the high viral loads and low CD4 counts <500 cells/mm<sup>3</sup> (current South African treatment guidelines) for ART showed a correlation (Table III and Fig. 1). Our analysis revealed low CD4 counts and viral loads for the first time in registered IDUs. To characterize the relationship of viral load response with the baseline CD4 cell count, three categories were defined in both cases. For the analysis of viral load, the ranges were classified (i) low <10,000 copies/ml, medium (10,000-99,999 copies/ml) and high (>100,000 copies/ml) viral load. Following the finer classification strategies, the percentage of low viral load with <10,000 copies/ml was about 3.8 of medium (Table III). In HCV and HIV infected individuals, both categories of medium and high viral load categories showed a significant percentage frequency depicting 19.23% and 76.92%, respectively (Table III). To evaluate the potential influence of HCV and HIV co-infection, a scatter plot analysis of all three infected viral categories was performed. The numeric representation of (intercept) HIV\_viral\_Load and HCV\_viral\_Load was 3.898e+02-1.680e-06 -2.534e-06 (Fig. 1). The mono-infected patients were less likely to establish a positive correlation between viral load and CD4 counts as the situation was vice versa in co-infected individuals. Both mono-infected correlations were less profound than in HCV and HIV co-infected patients.

Increased viral hepatitis particularly HCV infection has been a major health challenge in Pakistan for the past few years and its rising prevalence provokes a significant health burden on the concerned authorities for effective control (Rauf *et al.*, 2013). While several studies have been carried out on HCV infection alone, there has been limited investigations on HCV and HIV co-infection in Pakistan. This is of primary importance mainly due to the fact that HCV co-infection among IDUs is the major culprit of high mortality and morbidity in IDUs. The burden of HCV

**Table I. Prevalence of HIV, HCV, HBV and HCV-HIV co-infection in IDUs in four cities of Central Punjab.**

City	n	HIV positive	HCV positive	HBV positive	HCV-HIV co-infection	No viral infection
Lahore	104	14(13.46)	5(4.81)	0	84(80.77)	0
Sargodha	100	17(17.0)	4(4.0)	0	76(76.0)	1(1.0)
Faisalabad	83	12(14.46)	1(1.2)	0	68(81.93)	2(2.41)
Nankana	21	6(28.57)	1(4.76)	0	13(61.9)	1(4.76)
Total	308	49(15.91)	11(3.57)	0	241(78.25)	4(1.30)

**Table II. Percentage frequency of distribution of three viral co-infections among IDUs in Central Punjab.**

Total no. of individuals	HCV+HIV co-infection positive (%)	HBV+HIV co-infection positive (%)	HCV+HBV+HIV co-infection positive (%)
308	241 (78.25%)	1 (0.32%)	2 (0.65%)

**Table III. Frequency of distribution analysis of high viral load and low CD<sub>4</sub> count in HCV-HIV co-infected population of IDUs in Central Punjab.**

City	Viral load			CD4 count		
	Low (<10,000cp/ml)	Medium (10,000-99,999 cp/ml)	High (<100,000 cp/ml)	<200 x 10 <sup>6</sup> /L	200-349 x10 <sup>6</sup> /L	>350x10 <sup>6</sup> /L
Lahore	4(3.85%)	20(19.23%)	80(76.92%)	17(16.35%)	87(83.65%)	4(3.85%)
Sargodha	4(4.0%)	14(14%)	75(75%)	15(15%)	85(85%)	6(0.06%)
Faisalabad	3(3.61%)	14(16.87%)	66(79.52%)	9(10.84%)	74(89.16%)	5(6.02%)
Nankana	1(4.76%)	7(33.33%)	11(52.38%)	3(14.29%)	18(61.9%)	1(4.76%)
Total	12(3.90%)	55(17.85%)	232(75.32%)	44(14.29%)	264(78.25%)	16(5.19%)

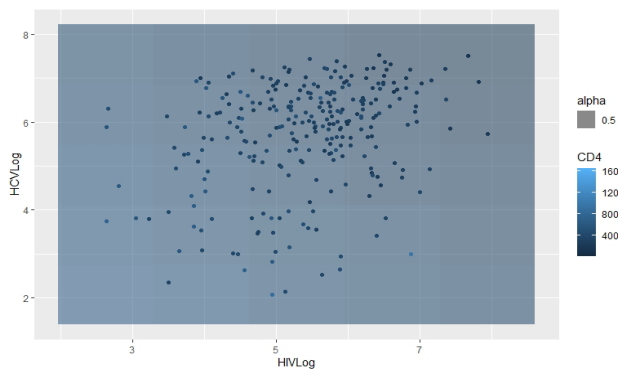


Fig. 1. Scatter plot for correlation between CD<sub>4</sub> and viral load of HIV and HCV monoinfected patients. The numeric representation of (intercept) HIV\_viral\_Load and HCV\_viral\_Load is 3.898e+02-1.680e-06 -2.534e-06. The regression line is a straight line demonstrating the best fit for measurement points on scatter plot. p-value is obtained by Pearson correlation tests.

infection in HIV infected individuals was reported to be high and needs to be addressed immediately. Pakistan being a developing country faces the challenges of high poverty index, illiteracy, and lack of proper awareness of HIV. Consequently, social taboos and religious beliefs do come in the way of proper education of viral infections such as HIV. Additionally, safe sex and lower socioeconomic status has contributed in worsening the situation of AIDS. According to myriads of studies, sharing of syringes among IDUs is a major culprit of co-infection and is far higher in developing countries (Todd *et al.*, 2007; Dumchev *et al.*, 2009; White *et al.*, 2007). Previously, it has been estimated that co-infection in Pakistan remained around 73%. However, recent studies performed by Mansha *et al.* (2017) have reported that this frequency has risen from 73% to 93% confirming the trend of using shared needles and syringes ultimately leading to high incidence of HCV co-infection in HIV infected IDUs in the country. Hence, the current study highlights the increase in frequency of HCV co-infection among HIV infected IDUs. A previous study reported the prevalence of HCV and HIV co-infection

in about 8.7% and HBV and HIV co-infection in about 7.8% of the Iranian population (Imani *et al.*, 2008). Direct blood to blood transmission is the most efficient means of transmission of both viruses (HIV and HCV). Prevalence analysis of HCV and HIV co-infections performed by Anwar *et al.* (2011) among jail inmates in Lahore observed a 9.19% ratio of the HCV positive inmates, and was much higher than HIV and HBV co-infection. Nevertheless, Jail inmates pose a very high-risk group and sustain high levels of HCV and HIV or HBV and HIV co-infections (Pontali and Ferrari, 2008; Vescio *et al.*, 2008). Similarly, HCV prevalence estimates among IDUs was also higher in multiple cities of Pakistan such as Rawalpindi and Quetta ranging from 17% to as high as 88% (Vickerman *et al.*, 2009; Kuo *et al.*, 2006). Research findings in Afghanistan also demonstrated synchrony showing a high prevalence of HIV, HCV and HBV infections in IDUs by common syringe usage in 50% of the IDUs and also by risky sexual practices (Todd *et al.*, 2007). Thus, these data analysis and trends indicate that HCV co-infection in IDUs is much higher than the generally anticipated and are comparable with the international data where prevalence of HCV and HIV co-infection reaches 100% in some countries (Kellerman *et al.*, 2003). Due to lack of information on HCV associated co-infections in general population or IDUs, the impact of HIV and HBV co-infections on the HCV epidemic in Pakistan needs to be monitored closely. Our study finding affirm the notion that IDUs remain on the vulnerable verge of acquiring opportunistic infections such as HCV and HIV co-infection but the transmission of HBV and HIV, individually or together, might be linked with unprotected sex both in heterosexuals and homosexuals. Moreover, Pakistan sustains approximately 10 million carriers of HCV and are more than HBV (Imran *et al.*, 2013). This study brings to light high prevalence of HCV infection in HIV infected IDUs.

It has been reported that the CD4 count is also reduced in the HCV and HIV infected population which correlates with the dilemma of reduced immunity and immune suppression in HIV infected population. In terms of advanced viral disease, CD4 cell count proves to be one of the most necessitated clinical management decisions in patients. A drop in CD4 count fewer than 200 cells/mm<sup>3</sup> indicates a critical threshold for increased risk of death (Hogg *et al.*, 2001). One of the most surprising findings do state the fact that HCV infection can also play a pivotal role in acquiring AIDS (Brennan *et al.*, 2016; d'Arminio *et al.*, 2009). In this research, we observed 83% of the HCV and HIV infected individuals carried low to medium reduction in CD4 count (200-349 x 10<sup>6</sup>/L) whereas 16.35% demonstrated very low CD4 counts (<200x 10<sup>6</sup>/L). These findings suggest that chronic viremia is associated

with both decreased levels of immune activation which suggests synchrony with other reported studies by Gonzalez *et al.* (2009). Although CD4 count analysis does help in the early diagnosis of HIV, however, the viral load estimation serves as a better diagnostic parameter for initiation of the ART treatment (Govender *et al.*, 2014). One of the limitations of this study was a small sample size of the mono-infected HIV and HCV individuals. However, the striking inference and revelation was of the CD4 correlation with viral loads in the HCV and HIV co-infected individuals particularly when limited studies have been conducted on co-infections in Pakistan. This study reports the overall viral load responses in all ranges and a strong positive correlation of CD4 and viral load in HCV and HIV co-infection, highlighting its novelty in unveiling the complications. This suggests the notion that CD4 may not only serve as a prognostic marker in diagnosis of HIV but it can also be used for diagnosis of HCV as we noticed CD4 levels drop in chronic HCV infection. The alarming situation of high frequency of HCV infection prevailing in HIV infected IDUs suggests dire need in understanding and awareness of the hazards caused by viral co-infections. The practice of sharing of syringes and high risky behaviours observed within the population of IDUs provokes the viral catastrophe, indicating some major responsibilities on the national and local bodies working for quality and safe management of health within the country.

## CONCLUSION

The prevalence of HCV infection was investigated in HIV infected IDUs in four major cities of Punjab Province of Pakistan. Our results provide key relevance of opportunistic infections such as HCV in HIV infected individuals. The HCV and HIV infections are predominantly associated with nonsexual parenteral route and relies on the transmission through blood borne routes. In this study, we report the shared use of syringes and needles as a major source of acquiring HCV and HIV co-infections. In co-infected individuals, a significantly decreased level of CD4 cells was seen in both medium high and very high viral load categories with a frequency of 19.23% and 76.92%, respectively. Taken together, current study highlights the increased prevalence of HCV co-infection in HIV infected IDUs with lower CD4 count. These results strongly support an immediate investigation of multiple viral infections routine IDUs to guide the eradication of these fatal viral diseases

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#### *Compliance with ethical standards*

This research study was ethically approved by the Institutional Review Board of University of Central Punjab and permitted by the Punjab AIDS Control Programme also.

#### *Statement of conflict of interest*

The authors have declared no conflict of interest.

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