



## Seroprevalence and comparison of three different screening tests for detection of human immunodeficiency virus infection in Peshawar, Pakistan

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### Abstract

Human immunodeficiency virus (HIV) concedes as a significant public health issue in the world from decades, which is growing speedily in number. Although, there seems to be insufficient data regarding HIV infection seroprevalence in general population of Peshawar, Pakistan. The objectives of the current study was to determine the seroprevalence of HIV infection in the general population of Peshawar, Pakistan and to compare the performance of three different screening methods for detection of HIV infection. A total of 5370 blood samples were screened for anti-HIV antibodies and HIV-RNA by using immunochromatographic test (ICT), enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) at Antiretroviral Therapy (ART) centre in Hayatabad Medical Complex (HMC), Peshawar. Out of 5370 blood samples, 756 (14.07%) were positive by ICT, 639 (11.89%) by ELISA and 606 (11.28%) by RT-PCR. The active HIV infection was high in males, i.e. 468 (13.61%) than in females 138 (7.14%). It was observed that infection was highest in the age group of 21–30 years that was about 237 (16.80%), whereas no sign of infection was observed for the age group of 01-10 years. However, the married population had an HIV seroprevalence of 423 (13.83%) while singles had 183 (7.91%) respectively. ELISA should be preferred for anti-HIV antibodies detection over ICT and RT-PCR should be preferred over ELISA for HIV-RNA detection. Moreover, the provision of treatment and screening facilities against this virus should be assured in medical care units of rural areas to prevent this infection.

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## Introduction

The human immunodeficiency virus is an enveloped lentivirus within the family of retrovirus. Its genetic material has a single nucleic acid structure known as a ribonucleic acid virus (Talarok and Talaro, 2002). It is one of the deadly epidemics that cause about 40 million infections worldwide (Alter, 2006). HIV could transmit from one person to another person through vaginal, anal or oral sex, blood transfusion, childbirth, contaminated hypodermic needles, breastfeeding and exchange between mother and child during pregnancy (Rothenberg *et al.*, 1998; Lee *et al.*, 2003). HIV damages the immune system of a person and as a result, causes acquired immunodeficiency syndrome (AIDS), which is a life-threatening and chronic disease. Worldwide, 25 million people are killed by HIV every year (Maartens *et al.*, 2014). At present, more than 40 million people worldwide are infected with HIV / AIDS and cause more than 3.1 million AIDS-related deaths each year (Valadas *et al.*, 2009). HIV infection has spread around the world. Millions of people are threatened with extinction. About 90% of people living with AIDS in developing countries live with illiteracy, poverty, underdevelopment, lack of medical facilities and hunger (Bhurri, 2006).

The three stages of HIV infection are acute, delayed and AIDS. The time and symptoms of each stage differ from the other stages. The duration of an acute infection is several weeks and can include symptoms such as esophageal mouth sores, malaise, muscle pain, swollen lymph nodes, mild temperature and lymphadenopathy. The duration of the late stage can last from 2 weeks to 20 years with little or no symptoms. The final stage, when CD4 count is less than 200, depends on an individual immune system called AIDS. The main symptoms of AIDS are shortness of breath and cough, diarrhea, rapid weight loss, vomiting, chronic fatigue, night sweats, nausea, repeated fever, sores in the nose or mouth, under the skin or on the genitals (Dieffenbach and Fauci, 2011). The major causes of HIV in Pakistan include unprotected sexual relations and the use of unsterilized syringes for injecting drugs. Moreover, low awareness about HIV and AIDS is also a reason

for the prevalence of this infection. HIV is considered to be a contagion infection which is being spread very speedily continuously (Ahmad and Khan, 2007; Maimaiti and Andersson, 2008; Jenness *et al.*, 2011). During this study, we examined anti-HIV antibodies and HIV-RNA in the general population of Peshawar, Pakistan. Our study aimed to find out the incidence of actual HIV infection and to compare the performance of screening techniques to find active HIV infection.

## Materials and methods

### *Study design and sample collection*

This retrospective study was carried out at Antiretroviral Therapy (ART) centre in Hayatabad Medical Complex (HMC), Peshawar from March 2017 to January 2018. In the present study, the total number of 5370 samples were collected from those individuals living in Peshawar and advised by health professionals for the screening of HIV infection. As Peshawar is the capital of province Khyber Pakhtunkhwa and by population, it is the 6th largest city in Pakistan and first most important city in the province. It has a population of 35.53 million and has covered an area of 74,521 km<sup>2</sup>. About three cubic centimeter blood was collected from each individual in a vacutainer tube, and HIV screening was performed using ICT, ELISA and RT-PCR based methods. The age groups of 03-79 years were included in this study.

### *Immuno-chromatographic tests (ICT)*

Serum was isolated from blood samples collected in vacutainer tube through centrifugation at high speed about 15000 revolutions per minute (rpm) for 5 minutes. Anti-HIV antibodies were detected by ICT method according to the manufacturer's instructions. The immune chromatographic strips (ICS) used were (Acon, USA) and (Accurate, USA).

### *Enzyme-linked immunosorbent assay (ELISA)*

ICT positive samples were tested for anti-HIV antibodies by ELISA using Biokit of Werfen Company (Spain) as described by manufacturers.

### *Nucleic acid extraction (NAE) and RT-PCR*

The confirmed ELISA positive samples were then subjected to HIV-RNA extraction by using Anagen RNA extraction kit (Anagen, USA) followed by RT-PCR. The kit of Norgen Biotek Corp (Canada) was used for RT-PCR as per manufacturer's instructions.

#### Statistical analysis

All the data was entered in Microsoft Excel sheet, and descriptive statistics were performed using the Statistical Package for the Social Sciences (SPSS), version 20.

#### Results

A total of 5370 blood samples were collected for this study, which included 3438 (64.02%) males and 1932

(35.97%) females. Furthermore, all the collected samples were labelled into seven age groups. Out of 5370 blood samples, 756 (14.07%) were positive for anti-HIV antibodies by ICT method. Moreover, ICT positive samples were confirmed by ELISA, and 639 (11.89%) were positive for anti-HIV antibodies. Furthermore, the ELISA positive samples were processed for HIV-RNA extraction through RT-PCR, and 606 (11.28%) were confirmed to be positive for HIV-RNA. The seroprevalence of active HIV infection was highest within the age group 21-30 years, 237 (16.80%) were found positive for the HIV infection while the lowest seroprevalence occurred within age groups 41-50 years, 39 (4.46%) were found positive for the HIV infection as shown in Table 1.

**Table 1.** Seroprevalence of HIV positive samples in relation to age groups.

Age groups	No. of samples	Anti-HIV +ve (by ICT)	(%)	Anti-HIV +ve (by ELISA)	(%)	HCV RNA +ve (by PCR)	(%)
01-10	351	-	-	-	-	-	-
11-20	1098	141	12.84	120	10.92	111	10.10
21-30	1410	282	20.0	243	17.23	237	16.80
31-40	1086	180	16.57	162	14.91	159	14.64
41-50	873	69	7.90	51	5.84	39	4.46
51-60	306	48	15.68	33	10.78	33	10.78
>60	246	36	14.63	30	12.19	27	10.97
Total	5370	756	14.07	639	11.89	606	11.28

HIV infection in male population was more prevalent 468 (13.61%) as compared to female 138 (7.14%). The result also shows a high seroprevalence of HIV

infection in the married population that is 423 (13.83%) while single was 183 (7.91%) respectively as shown in Table 2.

**Table 2.** Marital status and gender wise seroprevalence of HIV positive samples.

Gender	No. of samples	Anti-HCV +ve (by ICT)	(%)	Anti-HCV +ve (by ELISA)	(%)	HCV RNA +ve (by PCR)	(%)
Male	3438	534	15.53	486	14.13	468	13.61
Female	1932	195	10.09	153	7.91	138	7.14
Single	2313	240	10.37	192	8.30	183	7.91
Married	3057	489	15.99	447	14.62	423	13.83

#### Discussion

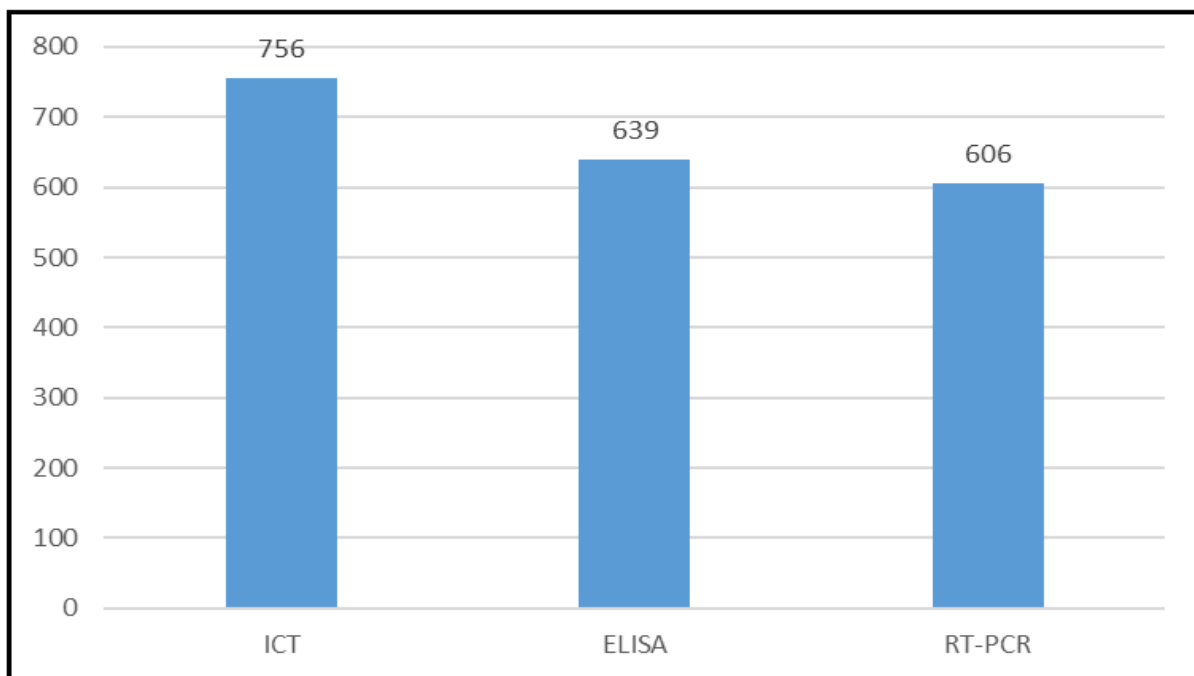
HIV is one of the major public health issue around the globe, especially in developing countries. Pakistan is defined as a high-risk country for the spread of HIV infection because it is situated between HIV high risks countries like Afghanistan on the west, India on

the east and China in the north. That is why Pakistanis get infected with HIV easily. Previous reports on HIV seroprevalence in other regions of Pakistan have shown high seroprevalence of HIV (27.27% and 27.3%) among the drug users and patients with renal disorders (Adil *et al.*, 2014; Altaf

*et al.*, 2016). Others regions like Sindh, Lahore, Rawalpindi, Punjab and Swabi has shown HIV seroprevalence of 22.25%, 2.01%, 21.6%, 5.3% and 4.50% respectively (Altaf *et al.*, 2009; Nafees *et al.*, 2011; Akhtar *et al.*, 2012; Shahid *et al.*, 2016; Zareen *et al.*, 2016).

Out of 5370 confirmed positive cases of HIV infection, 64% were males, and 36% were females. This majority of the male population is reported from around the globe in similar studies including Pakistan (Burnett *et al.*, 2005; Alter, 2006; Valadas *et al.*, 2009; Khattak *et al.*, 2014). High seroprevalence of

HIV infection was found in the married population. 15.99% samples were confirmed to be positive for HIV infection, and the same was reported from around the globe in similar studies including Pakistan (Burnett *et al.*, 2005; Zareen *et al.*, 2016; Azuonwu *et al.*, 2017). Divorced and widows were not included in our study. Amongst the age groups, the study showed that the infection is higher in the age group 21–30 years, followed by age group 31–40 years. This finding is in agreement with (UNICEF *et al.*, 2008), which shows that young people between the age group 15–24 years had a prevalence of more than 40%.



**Fig. 1.** Number of positive cases screened through different assays.

The seroprevalence of anti-HIV antibodies in this study was 14.07% when blood samples were screened through ICT devices, which is in agreement with the results of the studies mentioned above. We aimed to enhance the screening process so positive ICT serum was tested with the third generation of ELISA due to a false positivity rate of ICT devices (Mylonakis *et al.*, 2000; Junaid *et al.*, 2017). According to the ELISA test, 11.89% of blood samples were positive against anti-HIV antibodies. This result clears that ICT devices are not sufficient to reflect the true seroprevalence of HIV infection. ELISA is advised to

be more reliable than ICT devices. In many health care centers ICT devices are used to identify HIV infections. We suggested that ELISA should be put forward on ICT devices. ELISA can help to identify HIV core antigens in the blood. Developing countries such as Afghanistan, Bangladesh, India and Pakistan, which cannot afford to screen blood for nucleic acid amplification can take benefit from ELISA devices over ICT devices.

Regardless of whether there is an active infection or not, we examined the ELISA positive samples by using a real-time PCR test, which showed that 11.28%

of blood samples are actively infected HIV-RNA. A possible explanation for this reduction is the nature of disease, or the antibodies are present in the patient who is treated completely. The seroprevalence of active HIV infection in the general population of Peshawar, Pakistan is 11.28%. According to the previously published rates, this seroprevalence is relatively low. By using a suitable screening assay such as ELISA instead of an ICT device and PCR instead of ELISA can reduce the incidence of this viral infection.

### Conclusion

We conclude that the seroprevalence of active HIV infection in the general population of Peshawar, Pakistan is 11.28%. If we keep in view the previously published rates, this percentage is relatively low. The use of appropriate screening tests such as ELISA instead of ICT device, the incidence of this viral infection can be reduced.

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