ORIGINAL ARTICLE

Unrecognized HIV infection in asymptomatic volunteer blood donors at district Peshawar, Khyber Pakhtunkhwa, Pakistan

I. Haq[1], R. Ullah[2], M. Din[3], S. Ahmad[2], F. Anwar[1], M. Ali[3] and H. Ullah Khan[4,5]

1) *Department of Genetics, Hazara University Mansehra,* 2) *Department of Biotechnology, University of Malakand,* 3) *Department of Biotechnology, Quaid-i-Azam University Islamabad,* 4) *Khyber Medical College and* 5) *Khyber Teaching Hospital, Peshawar, Pakistan*

Abstract

Acquired immunodeficiency syndrome (AIDS) is a global epidemic that impacts the lives of many individuals each year. Human immunodeficiency virus (HIV) is a retrovirus that infects human $CD4^+$ T helper cells and macrophages thereby causing severe immune disease. The current study aimed to examine the prevalence of HIV among the blood donors of Khyber Pakhtunkhwa at Peshawar. In this study, a total of 8634 volunteers who donated blood were carefully screened for HIV using ELISA and RT-PCR techniques. Among the volunteers (n = 8634), 63 were positive by both ELISA and RT-PCR; which shows a prevalence of 0.73%. Both diagnostic techniques exhibited similar results. All the positive individuals were informed immediately and advised to start treatment to control the progression of the infection. It was concluded that HIV is on the rise in Peshawar, and routine screening and preventive measures are immediately required to address the urgent situation of HIV infection.

Keywords: Acquired immunodeficiency syndrome, ELISA, human immunodeficiency virus, Prevalence, RT-PCR, Viral infection
Original Submission: 11 December 2019; **Revised Submission:** 4 April 2020; **Accepted:** 15 April 2020
Article published online: 23 April 2020

Corresponding author: M. Din, Department of Biotechnology, Quaid-i-Azam University Islamabad, Pakistan.
Corresponding author: H. Ullah Khan, Khyber Medical College, Peshawar, Pakistan.
E-mails: misbah@bs.qau.edu.pk **(M. Din),** janaba555@gmail.com **(H.U. Khan)**

Introduction

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV), a member of the genus Lentivirus within the family *Retroviridae* that infects $CD4^+$ T helper cells and macrophages leading to severe immune suppression. In Pakistan, since the first reported diagnosis in 1987, the number of clinical cases of HIV infection has increased to 0.165 million [1,2]. The prevalence of HIV among the general population of Pakistan is estimated to be lower than 0.1%. As of 31 December 2019, the National AIDS Control Programme (NACP) had registered 36 902 individuals, an increase from the 4500 recorded in 2013 [2]. According to the NACP report, Punjab province has the largest number of HIV cases (75 000), followed by Sindh (60 000), Khyber Pakhtunkhwa (16 322) and Baluchistan (5275) [2]. Reports of such a large number of clinical cases from Punjab province compared with other provinces might be linked with the genetic diversity of HIV-1 subtypes and the prevalence of drug-resistance-associated substitutions. An alarming situation of HIV with high mortality was reported in Kot Imrana (Kot Momin), which is a small village located in the district of Sargodha in Punjab province [3]. In Karachi (Sindh province), a survey was conducted among injecting drug users in 2004 that confirmed more than 20% of them as HIV positive [4].

Prevalence of HIV in Pakistan is increasing among injecting drug users, and their sexual interactions may influence the increasing prevalence in the country [4]. The HIV epidemic in Pakistan is concentrated in key populations: people who inject drugs (38.4% of whom have HIV), male sex workers (5.6%), female sex workers (2.2%), transgender sex workers (7.5%), men who have sex with men (5.4%) and transgender people (7.1%) [5]. In a span of 10 years, reported HIV infections in

https://doi.org/10.1016/j.nmni.2020.100685

Pakistan increased from 8360 to 45 990 cases, the highest global average increase (17.6%) in history [6].

The Khyber Pakhtunkhwa health department reported 28 865 individuals with a sexually transmitted disease between 2011 and 2013, among whom very few received treatment [7]. Currently, the NACP reported 16 322 cases of HIV-positive individuals alone. Furthermore, the prevalence has been reported to be higher in male individuals compared with female individuals, which can be attributed to the lifestyle of men. The alarming increase in HIV prevalence may be a result of lack of awareness and improper protection against HIV infection [8].

There is a need for more intensive and focused awareness programmes regarding health education and safe injection practices in the country, particularly in Khyber Pakhtunkhwa province. This study aimed to examine the prevalence of HIV among blood donors in Peshawar Khyber Pakhtunkhwa, Pakistan.

Methods and materials

This study was conducted at the Khyber Teaching Hospital (KTH) Peshawar during the span of 6 months—June to November 2017. Informed consent was taken from all participating volunteers.

Samples collection and processing

During the study period, June to November 2017, blood samples were collected from a total of 8634 volunteers who donated blood to patients for different purposes from different geographic locations of Khyber Pakhtunkhwa at KTH Peshawar. Blood samples were taken from the blood donors and screened in the blood bank of KTH Peshawar. Blood was carefully screened for HIV using ELISA and RT-PCR techniques. The ELISA technique was used to assess HIV antigen/antibody in the blood samples and ELISA-positive individuals were further confirmed by RT-PCR.

Detection assays

ELISA Sandwich ELISA using the Human HIV Ag/Ab ELISA kit (Alere Determine; Abbott, Chicago, IL, USA) was performed to analyse the blood samples for either HIV antigen or antibody. Initially, sample distribution and identification plans were carefully established followed by preparation of a 1:20 dilution of the washing solution and reconstitution of the conjugate solution. Then, 100 µL of negative control was distributed to wells A1, B1 and C1 while 100 µL of positive control was added to well D1. Then, 100 µL of patient sample was added to wells E1, F1 and so on. Next, 50 µL of well-mixed conjugate solution was taken and the reaction mixture was homogenized. The micro-plate was covered with adhesive film and placed in a dark chamber for 1.5 hours at 37°C for incubation. The adhesive films were then removed, and the wells were decanted and washed with washing solutions. After that, 100 µL of freshly prepared development solution (substrate) was quickly dispensed into each well and kept in the dark for 30 minutes at room temperature. After development, 100 µL of stop solution was added to each well for 4 minutes. Finally, the optical density of the wells was recorded at 450 nm using a plate reader.

RNA isolation and RT-PCR After initial screening with ELISA, viral RNA was isolated from HIV-antigen-positive samples using artus® HI Virus-1 RG RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions [15]. The artus HI Virus-1 RG RT-PCR Kit constitutes a ready-to-use system for the detection of HIV-1 RNA using PCR. The HI Virus-1 RG Master A and B contains reagents and enzymes for the reverse transcription and specific amplification of a 93-nucleotide region of the 5′ long terminal repeat of the HIV-1 genome. Moreover, the specificity was validated with HIV-negative plasma samples (negative control) and external positive controls (HI Virus-1 RG QS).

Results

A total of 8634 people who donated blood voluntarily were screened for HIV antigen and/or antibody by ELISA. Among them, 63 were positive (0.73% prevalence) for HIV as antibody presence was confirmed in the serum using ELISA-based detection. The HIV-positive individuals were confirmed using RT-PCR, which is a comparatively more accurate and advanced diagnostic tool (represented in Fig. 1). Both techniques showed similar prevalence results, which were 0.73%. Furthermore, the number of individuals visiting hospital for blood donation in the 6 months under study was highest in August (1793), followed by July, and lowest in June (1103) (Fig. 1). Similarly, the chart also illustrates the percentage prevalence for the individual months June–November 2017, which was maximum in July and minimum in June. The prevalence observed month-wise was July > August > September > November > October > June.

Discussion

The increasing rate of HIV infection is a global health issue that has both social and economic implications. The first case of HIV infection in Pakistan was diagnosed in 1987, and numbers have been steadily increasing [1]. As a result of the lack of prevalence measures and knowledge about causative factors, HIV incidence has tended to rise in Pakistan at an alarming rate. In 2013, only 4500 individuals were reported as HIV positive, which had increased to 36 902 individuals in 2019 [5]. The diagnosis of

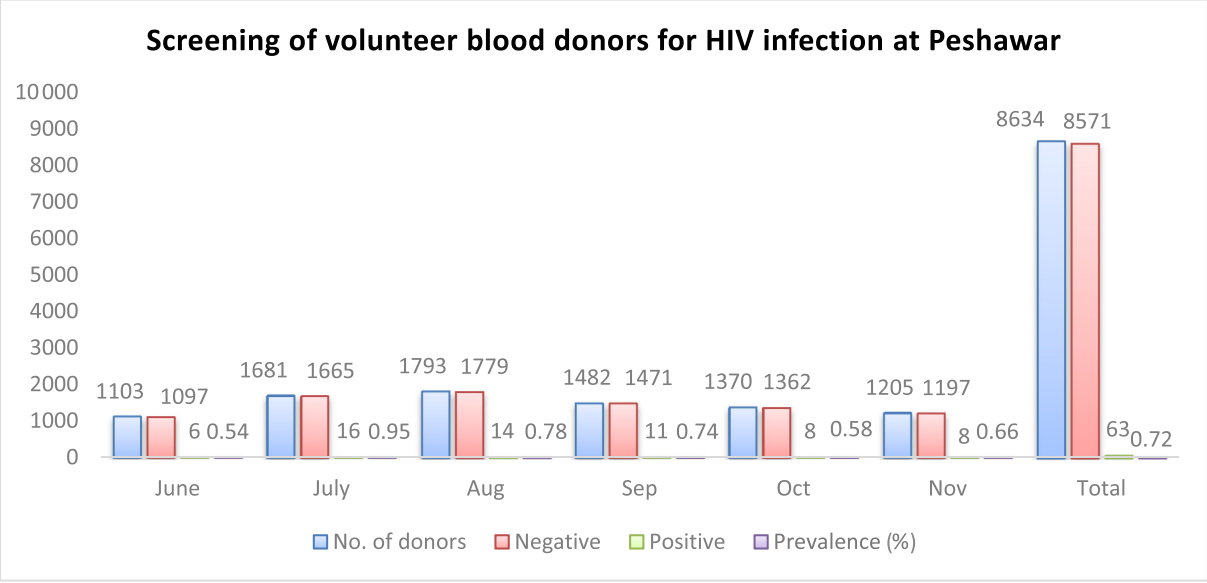

FIG. 1. Screening of volunteer blood donors for human immunodeficiency virus infection at Peshawar.

infection in its initial stages is very important in controlling HIV progression [9]. In 2003, the WHO survey in collaboration with the federal government of Pakistan reported a prevalence rate of 0.1% for HIV in individuals age from 15 to 49 years [10]. Prevalence rates of HIV infection in East Asia, Africa and South-east Asia are 25%, 15% and 10%, respectively [11]. The route of HIV transmission in Pakistan is heterosexual in 52.55%, and it has been reported that the most usual cause of HIV transmission is contaminated blood (11.73%) [12]. In 1992 and 1995, studies conducted by the NACP declared the prevalences of HIV infection to be 0.052% and 0.064%, respectively [4]. The HIV prevalence rate in 1998 among the general population in Lahore, Karachi, and Peshawar and Northern Pakistan was 0.1%, 0.06% and 0.5%, respectively [13].

The NACP has reported Punjab province to be the province most affected with HIV cases (75 000), followed by Sindh (60 000), Khyber Pakhtunkhwa (16 322) and Baluchistan (5275). Reports of more clinical cases from Punjab province compared with other provinces might be linked with the high population of the province and the genetic diversity of HIV-1 subtypes. An alarming incident of HIV with high mortality rate was reported in Kot Imrana (Kot Momin), which is a small village located in the district of Sargodha in Punjab province. In this village, among 5000 suspected individuals, about 669 screened positive for HIV in January 2019, which represents an increase from 1.29% to 13.38% in just 6 months [3]. In Larkana (Sindh province), 751 individuals were screened as positive for HIV among the total 26 041 individuals. Among the HIV-positive indivudlas, 604 were children living with HIV, which comprised 80% of the total positive cases [14]. Drivers identified by WHO for these outbreaks are unsafe blood transfusion practices, reuse of needles, male circumcision with unhygienic blades, and ear and nose piercing with unsafe needles [4]. The current scenario suggests that the overall prevalence of HIV in the general population is under-reported in Pakistan.

In our study, the blood donors were screened using ELISA to find the prevalence ratio for HIV-positive individuals of the KTH Peshawar, Pakistan. The serum of all the blood donors reporting to the KTH Peshawar, Pakistan from June 2017 to November 2017 was screened. In this study, screening of blood donors by ELISA revealed an alarming prevalence of 0.73%. This might be a result of the difference in the subject population, area of involvement or possibly seasons of study. In comparison with all the above studies conducted either in Khyber Pakhtunkhwa or in Pakistan, the tendency for HIV prevalence to rise was greater than previously thought. This might be a result of contamination in the health-care centre adopted by the patients or adopted for the patients in different hospitals, or might be due to a lack of awareness regarding spread of infection.

Conclusion

From our study, it can be concluded that HIV prevalence is increasing in Pakistan. We recommend the creation of awareness programmes, encouragement of protected sexual activity, developing restraining measures for injecting drug users/drug abuse and instituting routine blood screening. Preventive measures are immediately required to address this urgent HIV situation.

Ethical approval

The study was approved by the departmental ethics committee.

Animal rights

No animals were used for studies that are the basis of this research.

Conflict of interest

The authors declare no conflict of interest.

Authors contribution

MD and HUK contributed to conceptualization and methodology. IH and MD contributed to data curation, writing and original draft preparation. RU contributed to visualization and investigation. MA supervised the study and contributed to writing, reviewing and editing the paper. SA contributed to data collection and validation. FA contributed to writing, reviewing and editing the paper.

Acknowledgement

The authors are grateful to KTH staff for their help throughout the study.

References

[1] Khanani RM, Hafeez A, Rab SM, Rasheed S. Human immunodeficiency virus-associated disorders in Pakistan. AIDS Res Hum Retroviruses 1988;4:149–54.
[2] Pakistan National AIDS Control Programme. Available at: http://www.nacp.gov.pk/index.html. [Accessed 31 December 2019].
[3] Wahid B. An update on the severe outbreak of HIV in Kot Imrana, Pakistan. Lancet Infect Dis 2019;19(3):241.
[4] Bokhari A, Nizamani NM, Jackson DJ, Rehan NE, Rahman M, Muzaffar R, et al. HIV risk in Karachi and Lahore, Pakistan: an emerging epidemic in injecting and commercial sex networks. Int J STD AIDS 2007;18:486–92.
[5] Ahmed A, Hashmi FK, Khan GM. HIV outbreaks in Pakistan. Lancet HIV 2019;6(7):e418.
[6] Khan AA, Khan A. Sexually transmitted infection care in Pakistan: the providers perspective. J Pak Med Assoc 2012;62:941–5.
[7] Yusufzai A. Pakistan starts HIV treatment programme. BMJ 2006;332(7532):10.
[8] Khan A. HIV and homosexuality in Pakistan. Lancet Infect Dis 2009;9(4):204.
[9] Ullah AR, Hussain A, Ali I, Samad A, Shah ST, Yousef M, et al. A prospective observational study assessing the outcome of Sepsis in intensive care unit of a tertiary care hospital. Peshawar Pak J Med Sci 2016;32:688.
[10] Ilyas M, Asad S, Ali L, Shah M, Badar S, Sarwar MT, et al. A situational analysis of HIV and AIDS in Pakistan. Virol J 2011;8:191.
[11] Rai MA, Warraich HJ, Ali SH, Nerurkar VR. HIV/AIDS in Pakistan: the battle begins. Retrovirology 2007;4:22.
[12] Khawaja ZA, Gibney L, Ahmed AJ, Vermund SH. HIV/AIDS and its risk factors in Pakistan. AIDS 1997;11:843–8.
[13] Hyder AA, Khan OA. HIV/AIDS in Pakistan: the context and magnitude of an emerging threat. J Epidemiol Commun Health 1998;52:579–85.
[14] Altaf A. Delays and gaps in HIV programmes in Pakistan. Lancet HIV 2018;5:e678–9.
[15] artus® HI virus-1 RG RT-PCR kit handbook. Hilden, Germany: Qiagen; May 2011.