

# Human Immunodeficiency Virus Type-1 (HIV-1) Disease Progression and Viral Activity: A Seroepidemiological and Molecular Study

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## ABSTRACT

**Objective:** To determine the frequency and epidemiological characterization of *human immunodeficiency virus type-1* (HIV-1) infection, HIV disease progression, immune status and viral activity.

**Study Design:** Descriptive study.

**Place and Duration of Study:** Department of Microbiology, University of the Punjab and Institute of Public Health, Lahore, from September 2005 to August 2008.

**Methodology:** The study enrolled samples from general population, high risk groups and spouses of HIV+ deport workers with criteria; positive double enzyme linked immunosorbent assay (ELISA) and positive western blot. Immune status and viral activity was determined by cluster determinants (CD4+ and CD8+) cell count, ratio of CD4+/CD8+ on flow cytometer, and HIV RNA viral load on polymerase chain reaction (PCR).

**Results:** A total of 116 HIV+ untreated subjects enrolled after screening of 2260 blood samples. The seroprevalence rate in general population, high risk individuals and spouses of HIV+ deport workers was found 0%, 0.4% and 26% respectively. The CD4+ cell count was found 533/mm<sup>3</sup> (range 12-1800/mm<sup>3</sup>) and plasma viral load 27,122 copies/ml (range 00-40,621). The CD4+/CD8+ ratios < 0.5, < 1, < 1.5 and < 2 appeared as 17.2%, 30.2%, 51.7% and 0.9% respectively. Significant correlation was observed between plasma viral load, CD4+ count and CD4+/CD8+ ratio (p = 0.001). CD4+ T-cell counts < 200 cells/mm<sup>3</sup> was found in 23 HIV+ patients.

**Conclusion:** There was a low frequency of HIV in the general population and high risks groups as compared to very high frequency in spouses of HIV+ deport workers with significant correlation of viral activity and immune status.

**Key words:** HIV. CD4+/CD8+. Viral load. Epidemiology. Immunodepression. Spouse epidemiology.

## INTRODUCTION

The first *human immunodeficiency virus* (HIV)- positive Pakistani was identified in 1987.<sup>1</sup> Till March 2010, 3325 patients were registered at National AIDS Control Centre, NIH, Islamabad.<sup>2</sup> *Human immunodeficiency virus type 1* (HIV-1) specific CD8+ T-cells play a key role in the control of viral replication during HIV-1 infection. The cytotoxic T-lymphocyte (CTL) response is mainly measured at the early stage of infection and its appearance coincides with a rapid fall in plasma viremia during the early stage of infection with HIV-1.<sup>3</sup> Among all HIV high risk individuals in Pakistan, female sex workers (FSWs) formed the largest group reported, with estimates of 79127 and five different sub-typologies.

Injection drug users were the second largest group followed by male sex workers and hijra sex workers with estimates of 31555, 19320 and 14702, respectively.<sup>4</sup> The rationale behind the study was that in *human immunodeficiency virus type-1* (HIV-1) infection there is a decline in viral replication that has been attributed to host immunity, but the components of this response varies among individuals particularly the ability of cytotoxic T-lymphocytes to control viral burden and influence the outcome of the disease.

The objectives of the study were to determine HIV frequency in general population, high risk individuals for HIV and spouses of HIV+ deport Pakistani workers and to find out the immune status and plasma viremia with HIV disease progression.

## METHODOLOGY

It was a descriptive study with repeated cross-sectional surveys at fixed intervals on HIV+ patients followed longitudinally from September 2005 to August 2008. This study was conducted at Department of Bacteriology, Institute of Public Health and Microbiology Section of Faculty of Live Sciences University of the Punjab, Lahore. The technical assistance was gained from Armed Forces Institute of Pathology, Rawalpindi, and National Institute of Health, Islamabad. The study

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was approved by the Ethical Committee of the University of the Punjab and principles of Helsinki Declaration were followed. The consent form was prepared in Urdu. Those persons who did not sign the consent form were excluded from the study population in general as well as high risk individuals. The clinico-immunological progression of HIV disease was observed.

The subjects representing general population consisted of both sexes and all age groups residing in the surrounding area of Institute of Public Health, Lahore. Simple random sampling procedure was adopted by the study team comprised of one Doctor and two Laboratory Technicians of either gender. The questionnaire information related to HIV was solicited and the blood samples were collected according to format prepared prior to the sample collection. The standard precautions were followed during the blood extraction and clinical examination of the patients. The high risk individuals included were commercial sex workers (CSW) or prostitutes residing in Shahi Mohalla, Lahore. A simple random sampling was done on the basis of sample frame provided by the leader of the singing and dancing girl's union. Homosexual workers (HSW) were individuals who were reputed for indulging in homosexuality as passive partner,<sup>6</sup> contacted through their administrative chief (*Guru*). The third group was patients receiving multiple transfusions for haemoglobinopathies mainly thalassemia major and those leukemia contacted through Mayo and Services Hospitals, Lahore. The other groups were professional donors from various private clinics and volunteer donors registered in different private blood transfusion associations as well as intravenous drug users (IDUs) admitted in drug addiction control centres of Psychiatry units of Mayo and Services Hospitals and patients having generalized lymphadenopathy for more than 3 months in two or more extra inguinal sites were included. The spouses of Pakistani workers with HIV/AIDS deported from Gulf countries were included in the study through the Provincial AIDS Prevention and Control Centres. This group represents all provinces of Pakistan; Sindh, Punjab, Balochistan, Khyber Pakhtunkhwa including Federal Capital areas-Islamabad, and Azad Jammu and Kashmir (AJK).

The criterion for inclusion in the study population was; positive HIV on double ELISA and confirmed on Western blot. The manufacturer instructions (Abbott Murex) were followed for ELISA procedure. Lav. Blot 1 Ac- Ab-Ak kit (Diagnostic Pasteur, France) was used to detect HIV-antibodies in serum by immunoblotting (Western blot) in order to confirm the status of ELISA positive serum samples. The criteria of exclusion was those HIV+ patients who had received anti-retroviral treatment.

CD4+ and CD8+ T-lymphocytes count was performed on flow cytometer; FAC Scan Becton Dickinson Immunocytometry System 2350 Qune Drive San Jose, California 95131, (bench top).<sup>5</sup>

Blood samples were drawn aseptically by venepuncture into a sterile K3 EDTA (lavender top) vacutainer blood collection tubes. A minimum of 1 ml of whole blood was required. White blood cell count (WBC) and a differential cell count was obtained from the same sample of whole blood before staining. Sysmex KX-21N Automated Haematology Analyzer was used for white blood cell (WBC) count. An acceptable WBC concentration range for the method used was described to be ranging from  $3.5 \times 10^3$  to  $9.4 \times 10^3$  WBC/ $\mu$ l. Samples with counts greater than  $9.4 \times 10^3$  WBC/ $\mu$ l were diluted with IX phosphate-buffered saline (PBS) containing 0.1% sodium azide, or cell wash. For the samples with less than  $3.5 \times 10^3$  WBC/ $\mu$ l, more blood was required. Blood was stained with reagent A through F within 6 hours after drawn (Becton Dickinson catalog No. 349524). For the percent lymphocyte, conversion software option was selected, which automatically calculated each reported lymphocyte subset as a percentage of lymphocytes in the lymphocyte analysis gate.

Viral load was determined by quantification of viral RNA in peripheral blood by RT-PCR (reverse-transcription PCR) on Roche Diagnostics System which comprised of the following four equipments; COBAS Amplicor PCR analyzer-Roche Diagnostic; COBAS AmpliPrep™ analyzer-Roche Diagnostic; Amplilink PC; and Barcode scanner.<sup>7</sup> The results were categorized in four groups as < 500, 500 to 10,000, 10,001 to 100,000 and > 100,000 copies/ml.

Statistical Package for Social Sciences (SPSS) statistical software version 10 was used. Descriptive statistics including 95% confidence interval between upper and lower limit-Confidence Interval (CI) were determined. Pearson correlation was considered significant at the 0.01 level (2-tailed). Continuous variables were summarized using median, mean and standard deviation (SD) and categorical variables using frequencies and percentages. The chi-square test was used for comparing proportions.

## RESULTS

Of the 2260 total individuals tested for HIV, 1050 (46.46%) belonged to the general population, 1010 (44.69%) represented high-risk groups and 200 (8.85%) spouse of HIV+ deported workers. The seroprevalence rate of HIV in general population, high-risk individuals and spouses of deported workers was found 0%, 0.4% and 26% respectively. The mode of transmission among HIV+ individuals was as follows; heterosexual 75 (64.7%), homosexual transmission 2 (1.75%), IDUs 4 (3.4%), blood transfusion 12 (10.3%) and unknown 23 (19.8%). The area wise distribution of HIV+/AIDS cases was as follows. Federal/Islamabad in 14 (12.1%), Punjab in 33 (28.4%), Sindh in 47 (40.5%), Khyber Pakhtunkhwa in 16 (13.79%), Balochistan in 5 (4.3%) and AJK in one (0.9%) case.

**Table I:** Clinical positive findings in HIV/AIDS cases.

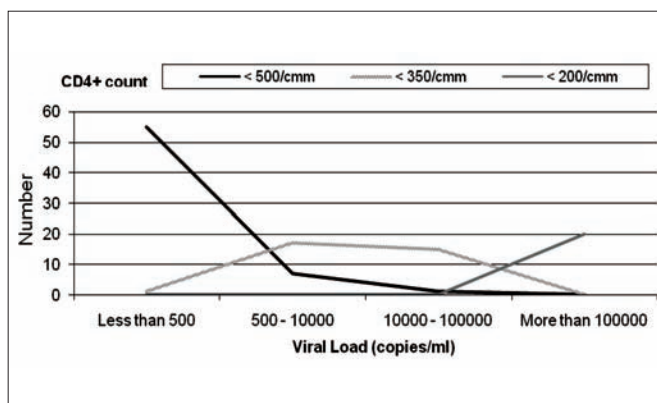
Symptoms / Signs / Diagnostic	Number of positive HIV+ cases (%)						Total
	Federal area	Punjab	Sindh	Khyber Pakhtunkhwa	Balochistan	AJK	
Kaposi sarcoma	0	0	0	0	0	0	0
Disseminated/ extrapulmonary / non-cavity pulmonary tuberculosis	0	0	0	0	0	0	0
Oral candidiasis / hairy leukoplasia	1 (0.86)	3 (2.59)	8 (6.90)	1 (0.86)	0	0	13 (11.2)
Pulmonary tuberculosis with cavitations, or unspecified	0	0	4 (3.4)	0	0	0	4 (3.4)
Herpes zoster in a person of 60 years or less	0	0	3 (2.6)	0	0	0	3 (2.6)
Central nervous system dysfunction	0	0	0	0	0	0	0
Diarrhea one month or more	1 (0.86)	3 (2.59)	6 (5.17)	0	0	0	10 (8.6)
Fever of at least 38°C for at least a month	1 (0.86)	6 (5.17)	4 (3.45)	1 (0.86)	0	0	12 (10.3)
Cachexia or weight loss of more than 10%	1 (0.86)	4 (3.45)	6 (5.17)	1 (0.86)	0	0	12 (10.3)
Asthenia of at least a month	0	0	0	0	0	0	0
Persistent dermatitis	0	3 (2.59)	7 (6.03)	0	0	0	10 (8.6)
Anaemia, lymphopenia, and/or thrombocytopenia	0	4 (3.45)	10 (8.62)	1 (0.86)	0	0	15 (12.9)
Persistent cough or any pneumonia, and/or thrombocytopenia	0	0	0	0	0	0	0
Lymphadenopathy of at least two non-inguinal sites	0	2 (1.72)	7 (6.03)	0	0	0	9 (7.8)

Acute retroviral syndrome like mild fever, lethargy, malaise and headache was found in 37 cases (31.87%); 56 (48.3%) were asymptomatic. The clinical findings among the 116 HIV/AIDS cases are given in Table I. Immunologically HIV disease progression was categorized into five stages; asymptomatic primary in 2 (1.7%), symptomatic primary in one (0.9%), asymptomatic latent in 49 (42.2%), symptomatic latent in 41 (35.3%) and AIDS in 23 cases (19.8%). During the follow-up examinations 20 cases (17.24%) of the 116 HIV/AIDS cases developed opportunistic infections. These included pulmonary tuberculosis in 4 (3.4%), oral candidiasis in 13 (11.2%), and herpes zoster in 3 (2.6%) cases. Patients characterized with CD4+ lymphocyte counts < 200/ml and CD4+ percentages < 14% were presented with opportunistic infections. Patients with a viral load over 10,000 copies/ml also had a more frequent incidence of opportunistic infections. In general, the high viral load was associated with more frequent opportunistic infections, regardless of respective CD4+ lymphocytes' level. Four of the HIV/AIDS patients (3.4%) had associated infection (co-infection). Three subjects were found hepatitis B (HbsAg) positive, while anti HCV appeared in one case. Six HIV/AIDS patients (5.2%) showed sexually transmitted diseases other than HIV. Syphilis was diagnosed in 4 of the cases following screening test, haemagglutination (TPHA) for *Trypanema pallidum* test. Similarly, gonorrhoea was diagnosed on the basis of typical history of urethral discharges in two of the seropositive HIV individuals, which was confirmed by examining direct Gram's stained smears.

The distribution of CD4+ cell count among 116 HIV+/AIDS patients was as follows; patients presented with cell counts < 500/mm<sup>3</sup>, < 350/mm<sup>3</sup> and < 200/mm<sup>3</sup> were 63 (54.3%), 33 (28.4%) and 20 (17.2%) respec-

**Table II:** CD4+/CD8+ ratios amongst the HIV positive and AIDS patients.

CD4+/CD8+ ratio category	HIV disease status				p-value value
	HIV positive cases		AIDS cases		
	No.	%	No.	%	
< 0.5	0	0	20	17.2	< 0.0001
< 1.0	34	29.31	01	0.86	0.0019
< 1.5	58	50.00	02	1.72	< 0.0001
< 2.0	01	0.86	0	0	0.617
Total	93	80.17	23	19.78	-



**Figure 1:** Viral load (copies/ml) and CD4+ (cells/cmm) count among HIV+/AIDS patients.

tively. All above patients were tested again for CD4+ cell count after 6 months and the count remained the same in three groups mentioned above. Of total 116 HIV+/AIDS patients, 56 (48.3%) showed viral load < 500 copies/ml; 24 (20.7%) 500-10,000 copies/ml; 24 (20.7%) 10,001-100,000 copies/ml and 20 (17.2%) > 100,000 copies/ml. Figure 1 shows the description of viral load (copies/ml) and CD4+ count (cells/cmm) among HIV+/AIDS patients. The distribution of CD4+/CD8+ ratios amongst the HIV positive and AIDS patients is given in Table II.



## DISCUSSION

International labour migration, or the movement of people across national borders for employment, is a growing phenomenon and an increasingly important aspect of global, regional and national economies. However, HIV has become a key issue of concern with cross border and overseas migration. In the present study highest prevalence rate (26%) of HIV infection was found among the spouses of deported Pakistani workers; a similar figure was reported in other study.<sup>8</sup> Pakistan has high rate of external migration. Mobility and migration themselves are not risk factors for HIV, but they may lead to situation in which people may become vulnerable to the infection; separation from spouse, family, society and traditions, together with isolation, loneliness and a sense of anonymity can lead to unsocial and sexual practices, which may increase the exposure to HIV. This group may be a major threat of HIV epidemic in Pakistan and this can indirectly be proved that all clients of HIV clinics in Pakistan are the expatriated migrant workers and their immediate relations.<sup>9</sup> We found that a large number of deport HIV+ Pakistani workers (82%) were returned from the Gulf countries similar to other studies.<sup>10</sup> The above workers were screened there on routine investigations, which are mandatory for renewal of contract and resident permit.

In the current study, HIV frequency in general population and high risk population was found low which may be due to social, cultural and religious impact on the risk for contracting HIV disease in Pakistan. Very low prevalence of HIV among CSW, HSW and other high risk group was comparable to other reports.<sup>11-13</sup> The characteristics of HIV-specific immune responses and the parameters of HIV infection in Pakistani population is not fully understood because of the non-availability of data. The relationship between HIV-specific T-lymphocyte response and viral replication was studied among Pakistanis who are infected with HIV-1. High viral load was found in a significant number of HIV+ patients which later developed symptoms of AIDS and was considered as an indicator of the disease's progression.<sup>14,15</sup> There were 23 HIV+ symptomatic patients in this study who showed high viral load and rapidly decreasing CD4+ count < 200/mm<sup>3</sup> and urgently seeking antiretroviral treatment,<sup>16</sup> but unfortunately none was received during the study period. HIV+ patients had elevated numbers of CD8+ cells during early infection, which was considered as a viral set point determination on HIV disease progression.<sup>17</sup> The influence of CD8+ T-lymphocyte function on HIV disease progression is of considerable interest as cytotoxic T-lymphocytes (CTLs) are the main effector cells of the specific cellular immune response. Activated by CD4+ T-helper cells, anti-HIV specific CD8+ T-cells have a crucial role to play in the control of viremia,<sup>18-20</sup> and increased in response to ongoing viral replication.<sup>21</sup>

## CONCLUSION

A low frequency of HIV was found among the general population and the high-risk groups while it was higher among the spouse group of HIV+ deport workers. The majority of deport workers returned from the Gulf countries and appeared a major threat of HIV disease in Pakistan. High viral load and low total CD4+ T-lymphocyte count were considered to be one of the main indicators of the progression of HIV-induced immunodepression in patients.

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