

# Seroprevalence of Human T-Cell Lymphotropic Virus-1/2 in Blood Donors in Northern Pakistan: Implications for Blood Donor Screening

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## ABSTRACT

**Objective:** To determine the seroprevalence of Human T-cell Lymphotropic Virus-1/2 (HTLV-1/2) in blood donors in Northern Pakistan.

**Study Design:** Descriptive study.

**Place and Duration of Study:** Armed Forces Institute of Transfusion, Rawalpindi, from July to August 2013.

**Methodology:** A total of 2100 blood donors were screened for anti-HTLV-1/2 antibodies during the study period, in a pool of six, on a highly sensitive, Chemiluminiscent Microparticle Immunoassay (CMIA) based system. The screening test-reactive donors were recalled, counseled and interviewed, and a fresh sample was obtained for confirmatory testing. Confirmation was performed using additional immunoassays including Line Immunoassay (LIA); with additional testing for HTLV-1 pvDNA PCR. Frequency and percentages were determined.

**Results:** Four donors (0.19%) were repeatedly screening test-reactive and were subsequently confirmed to be HTLV-1 infected by line immunoassay and HTLV-1 pvDNA PCR. All four donors were male with mean age of 27 ±6.27 years. Two (50%) of the positive donors gave history of Multiple Sexual Partners (MSP).

**Conclusion:** HTLV-1 seroprevalence in Northern Pakistan blood donors was determined to be 0.19%. Large scale studies, including the cost effectiveness of screening blood donations for anti-HTLV-1/2 in Pakistan, are recommended.

**Key Words:** Seroprevalence. HTLV-1/2. Blood donors. Pakistan.

## INTRODUCTION

Human T-cell Lymphotropic Virus-1 (HTLV-1) has been identified as the causative agent of Adult T-cell Leukemia/Lymphoma (ATLL).<sup>1</sup> It has also been identified as a cause of HTLV-1 associated myelopathy (HAM) or Tropical Spastic Paraparesis (TSP), a chronic debilitating neurological condition.<sup>2</sup> The lifelong risk in an infected carrier for development of ATLL is estimated at 1 - 5% over a period of 20 years and at 4% for HAM/TSP over a shorter period of time.<sup>3</sup> HTLV-3 and HTLV-4 were discovered in 2005 in Africa but, as with HTLV-2, currently no specific disease associations have been attributed to these viruses.<sup>4</sup>

HTLV-1 is endemic in southern Japan, south and central America, central Africa and northeastern Iran.<sup>5-8</sup> However, the distribution of HTLV-2 remains restricted amongst Injection Drug Users (IDU) and certain native communities of central and south America. One of the main routes of transmission for HTLV-1/2 is the transfusion of infected blood products.<sup>9</sup> Therefore, screening of blood donations for anti-HTLV-1/2 antibodies has been introduced by a number of developed

countries over the last 10 - 15 years.<sup>10</sup> The decision to implement HTLV-1/2 for blood products screening depends upon the prevalence and incidence of HTLV, but is also influenced by the available resources. However, there is no reliable HTLV-1/2 seroprevalence data for Pakistan which would help inform decision making about any possible need for HTLV-1/2 screening of blood donations.

This study was, therefore, conducted to provide initial data, with the aim of determining the seroprevalence of HTLV-1/2 in blood donors in Northern Pakistan.

## METHODOLOGY

All blood donations collected at the Armed Forces Institute of Transfusion (AFIT), from July to August 2013 were included. Non-probability convenience sampling was done. The study was approved by the Ethical Committee of AFIT. All the donors provided informed consent before inclusion in the study. Prior to blood donation, the donors were assessed for their suitability to donate by following the standard donor selection guidelines of the Institute. All the donors not fulfilling the required standards of donor selection like age, weight, hemoglobin levels, history of hepatitis B, hepatitis C or HIV positive test result, history of fever or dental procedure in the past one week, were excluded from the study. All donors provided the standard informed consent before donation and sample collection. Serum samples were collected from the donors in serum separator tubes.

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Samples collected from the donors were tested in pools of six, pooling being performed using a Hamilton STAR IVD Pipettor (Hamilton, Bonaduz, Switzerland). The pooled samples were screened for anti-HTLV-1/2 antibodies using the rHTLV-I/II assay run on an Abbott ARCHITECT i2000 system (Abbott Diagnostics, Abbott Park, IL). Initial reactive pools were resolved by testing the individual donations to identify the reactive donor within the pool of six. Donations were released or discarded on the basis of the anti-HTLV-1/2 screening results. Confirmatory testing of anti-HTLV-1/2 antibody reactive donors was not available at the study centre but was provided by the National Transfusion Microbiology Reference Laboratory (NTMRL), London, England. The standard HTLV serology confirmatory algorithm was followed, comprising two alternate anti-HTLV-I/II microplate immunoassays (Innotest HTLV Ab IV, Fujirebio, Ghent, Belgium; and HTLV Ultra Diapro, Milan, Italy) followed by an HTLV-I/II line immunoassay (LIA) (Inno-Lia HTLV-I/II Score, Fujirebio, Ghent, Belgium). Additionally, HTLV-1 pvDNA PCR was performed in an in-house Taqman PCR, specifically designed for HTLV confirmation in NTMRL.

All HTLV-screen reactive donors were recalled, notified of their anti-HTLV-1/2 screen results, and counseled. They were also deferred from donating blood in future. On receipt of the confirmatory results, the reactive donors were recalled again and the results of the confirmatory tests were communicated to them. The donors were interviewed about several risk factors for transmission of HTLV-1/2 infection. The risk factors included Multiple Sexual Partners (MSP), blood transfusion, travel to endemic areas, travel to endemic areas by the parents of the donors, diagnosis or death of parents by leukemia or Injection Drug Use (IDU) in the past. Confirmed positive donors were directed to periodical visits by appropriate clinical specialists for ongoing care. The screening algorithm that we prepared and followed in this study is shown in Figure 1.

Statistical Package for Social Sciences (SPSS) version 17 was used for statistical analysis of the data. Frequency and percentage was calculated for all the

qualitative variables like gender and anti-HTLV-1/2 test positive status. Mean value  $\pm$ SD was calculated for quantitative variables like age.

## RESULTS

A total of 2100 routine blood donations (350 pools) were screened for anti-HTLV-1/2. Four pools were found to be initially screening test reactive. Individual testing of the pool members identified 4 (0.19%) HTLV-1 reactive donations out of a total 2100 tested. All 4 (100%) of these donors were serologically confirmed to be HTLV-1 by NTMRL, for all the specific bands on the LIA (p19 I/II, p24 I/II, gp46 I/II, gp21 I/II, p19 I, gp46 I). The bands, found positive by LIA assay for the four reactive samples have been summarized in Table I. Therefore, seroprevalence of HTLV-1 among the blood donors during the course of this study was 0.19%. Follow-up samples were obtained from these reactive donors, and all 4 (100%) tested positive for the presence of HTLV-1 pvDNA by PCR, further consolidating initial results.

Of the 2100 donors, 2067 (98.4%) were male and 33 (1.6%) female, with a mean age of  $29 \pm 9.26$  years, (ranging from 18 - 60 years). The basic demographic data of four positive donors is presented in Table II. All 4 donors were referred for specialist advice by hematologist and neurologist.

Two of the positive donors provided a history of multiple sexual partners but no other risk factors were identified in any of the four positive donors. None of these donors provided any history of clinical symptoms associated with HTLV-1/2 disease and all were asymptomatic, both at the time of donation and recall/counselling sessions. All of the other standard infectious disease screening tests were applied to the blood donations; HBsAg, anti-HCV, HIV antigen/antibody and syphilis antibodies were negative. One of the positive blood donors was married and after the counselling session, he was given the option to get his spouse tested for anti-HTLV-1/2. The spouse, subsequently, also tested positive for anti-HTLV-1/2 antibodies. She was also counseled along with her husband.

**Table I:** Line immunoassay (LIA) confirmatory patterns of four positive donors.

| Donor no | p19 I/II | p24 I/II | gp46 I/II | gp21 I/II | p19 I | gp46 I | gp46 II | Remarks                                      |
|----------|----------|----------|-----------|-----------|-------|--------|---------|--|
| 001      | 3+       | 2+       | 3+        | 3+        | 3+    | 3+     | -       | Consistent with HTLV-I infection in the past |
| 002      | 2+       | 2+       | 3+        | 3+        | 2+    | 3+     | -       | Consistent with HTLV-I infection in the past |
| 003      | 3+       | 2+       | 3+        | 3+        | 3+    | 3+     | -       | Consistent with HTLV-I infection in the past |
| 004      | 2+       | 3+       | 3+        | 3+        | 2+    | 3+     | -       | Consistent with HTLV-I infection in the past |

**Table II:** Epidemiological data of the HTLV-I/II positive donors.

| Donor no | Age (years) | Gender | Risk factors | HTLV-Associated disease | HBsAg    | Anti-HCV | HIV      | Syphilis |
|----------|-------------|--------|--------------|-------------------------|----------|----------|----------|----------|
| 001      | 34          | Male   | MSP*         | None                    | Negative | Negative | Negative | Negative |
| 002      | 19          | Male   | None         | None                    | Negative | Negative | Negative | Negative |
| 003      | 29          | Male   | MSP          | None                    | Negative | Negative | Negative | Negative |
| 004      | 26          | Male   | None         | None                    | Negative | Negative | Negative | Negative |

\* MSP: Multiple sexual partners

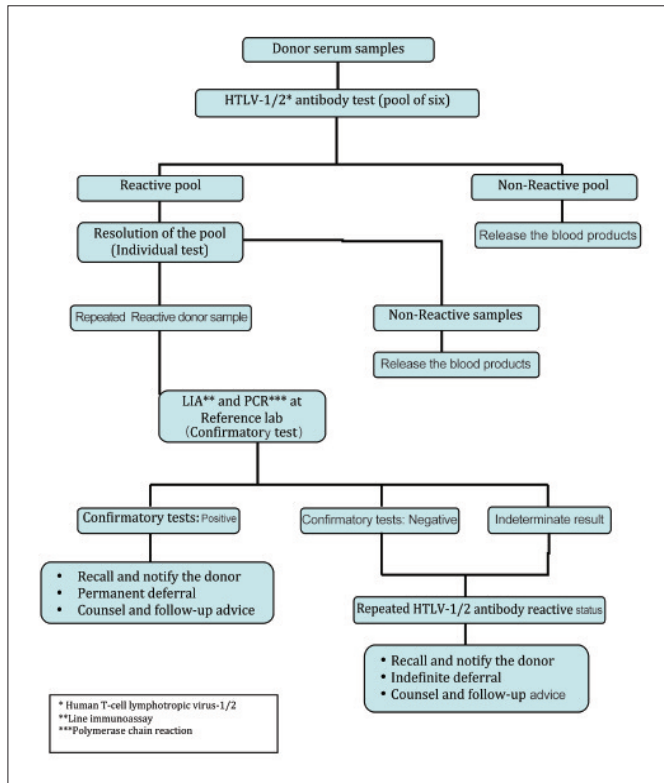


Figure 1: Algorithm for HTLV-1/2 screening.

### DISCUSSION

There is no previously published HTLV prevalence data for Pakistan, although there are some local studies in the geographical area which are available for comparison. The researchers screened the blood donors in pools of six in this study. Several studies in the past have evaluated the strategy of pooling the serum samples for anti-HTLV-1/2 screening and found this to be appropriate, as in general HTLV antibodies tend to be both high titre and high avidity, allowing pooled screening without significant loss of sensitivity.<sup>11,12</sup>

The overall seroprevalence, from this study, was 0.19%, in contrast to the reported prevalence of HTLV-1/2 from the other countries in the region. In Saudi Arabia, 3/47,426 (0.006%) blood donations were identified as HTLV-1 positive, although only 1 of these positive donors was a native Saudi, the other 2 were Indian expatriates.<sup>13</sup> In China, the reported HTLV prevalence in blood donors is 0.06%.<sup>14</sup> In comparison to this region, HTLV-1 infection is quite uncommon in blood donors from USA and Western Europe.<sup>15,16</sup> Higher seroprevalence rates among blood donors have been reported from some parts of the world, highlighting the microgeographic clustering of infected individuals across the globe.<sup>17</sup> In this study, all the four positive donors had antibodies against HTLV-1 which was independently confirmed by Western-blot analysis and by HTLV-1 pDNA testing. Most of the studies conducted in our region only detected HTLV-1 as the assay used

did not include anti-HTLV-2 testing.<sup>8,14</sup> HTLV-1 is generally the more prevalent virus type in UK and Europe, whereas the rate of infection with HTLV-2 is higher than HTLV-1 in USA and South America.<sup>16-18</sup>

Studies conducted in the past have shown that a significant correlation exists between increasing age and prevalence of infection with HTLV-1.<sup>8,15</sup> The positive individuals in this study were all young adults with a mean age of 27 years, 2 years less than the mean of all the donors in our study. As the age of positive donors ranged between 19 to 34 years and the number of positive donors is also quite low, the pattern of gradual increase with age in seroprevalence could not be established. It is also well established that the seroprevalence of HTLV-1 is higher in the females than males.<sup>15,19</sup> All the positive donors were males, due to an overall disproportionate high numbers of male blood donors at our institute, and across Pakistan as a whole.

There is sufficient evidence to support the fact that seroprevalence rates may be different in different provinces or states within the same country.<sup>18</sup> A high prevalence in a certain province or state within a country would certainly require initiation of screening program in that particular province or state if not the whole of the country. This is currently the situation in Iran where blood is screened for HTLV-1 in the Great Khorasan province only, where the HTLV-1 seroprevalence is quite high.<sup>8</sup> There is a need to conduct large scale studies in future to look into the regional differences of HTLV seroprevalence within Pakistan. There also appears to be an association of HTLV infection with hepatitis B and hepatitis C. An 11-year follow-up study conducted in Brazil highlighted the importance of HTLV co-infection with hepatitis B virus and hepatitis C virus.<sup>20</sup> Similarly, another study has identified anti-HBc as an independent risk factor for HTLV-1 infection.<sup>21</sup> Such co-infections were not identified in any of the positive donors in this study.

Transfusion of unscreened blood has been recognized as one of the routes of HTLV transmission. The rate of transmission of the virus to the recipient after transfusion with cellular components is more than 40%. Some case reports in the past have documented rapid development of myelopathy after infection with HTLV-1 acquired by transfusion.<sup>22</sup> Introduction of a screening program for HTLV has resulted in decrease in the prevalence of this virus among the blood donors in every country in which it was introduced. HTLV-1 seroprevalence in blood donors in highly endemic country like Japan has declined steadily since 1988 onwards.<sup>23</sup> Similarly, a reduction in the prevalence among US blood donors has also been observed since early nineties.<sup>15</sup> Most of the developed countries have been carrying out screening for HTLV-1/2 and reaping the benefits of reducing the HTLV-carrier burden over the years, whereas the need for such screening has been overlooked in the less developed countries like Pakistan, primarily due to financial constraints.



The question then remains as to whether it would be beneficial to screen the blood donations in Pakistan for anti-HTLV-1/2? The answer to this question lies in the availability of data about HTLV-1/2 seroprevalence in Pakistan, the likelihood of transmission through the components transfused and the provision of sufficient financial resources for screening and the confirmation of screen reactive donors. The literature is replete with studies about the implementation of screening of blood donations for HTLV-1/2 in developed countries, reporting seroprevalences which are much lower than that in our study. In USA, the seroprevalence of HTLV-1 is 5.1/100,000 (0.005%) where the screening has been going on for more than two decades.<sup>15</sup> The UK with a seroprevalence even lower in the first time blood donors i.e. 4/100,000 (0.004%), started screening its blood products in 2002.<sup>17</sup>

### CONCLUSION

The seroprevalence of HTLV-1/2 in Pakistani blood donors in this study was relatively high at 0.19%. This indicates a potential problem with the transmission of HTLV through transfusion, possibly at a high rate. Large scale studies are required across whole of the country, to properly ascertain the national prevalence of HTLV and consequently determine both the clinical need and operational feasibility of implementing such screening. This study will also serve as the basis for the future monitoring of an increase or decrease in the prevalence of HTLV-1/2 in Pakistani blood donors.

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