Seroprevalence of human T-lymphotropic virus antibodies among patients with lymphoid malignancies at a tertiary center in Lagos, Nigeria

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Background: There is a significant association of Human T-lymphotropic viruses (HTLV) with lymphoid malignancies. HTLV causes lymphoma/malignancy of CD-activated cells (HTLV-1-associated lymphoma/myeloma (ATL), and chronic myeloproliferative syndrome called adult T-cell leukemia/lymphoma (ATL) and Chronic Myeloproliferative Syndrome called tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM)). This study aims to determine the prevalence of HTLV-1 among patients with lymphoid malignances (ATL, TSP/HAM) at a tertiary center in Lagos, Nigeria.

Methods: A cross-sectional study was carried out at the hematology clinic of the Lagos State University Teaching Hospital. A total of 30 patients with lymphoid malignances were recruited from each subject. The serum was separated and stored at -18°C. This study was done in the Department of Hematology and Blood Transfusion, Faculty of Clinical Sciences, College of Medicine, Lagos State University, Lagos State, Nigeria.

Results: A total of 30 patients with lymphoid malignancies were reactive on the ELISA test. On confirmatory testing with Western blot confirmatory testing, only two patients (6.7%) were positive for HTLV-1 and 18 patients (60%) were positive for HTLV-2. Western blot confirmatory testing was done on reactive samples. All patients were divided into two groups: patients with malignancies and patients with HIV. There was no association between history of previous blood transfusion and positivity to HTLV-1/2 (P=0.544).

Conclusion: A prevalence of 5.1% of HTLV-1 among patients with lymphoid malignancies was found in this study. The patients were younger, more males, and HIV-negative.

Keywords: HTLV, Lymphoid malignancies, ATL, ELISA, TSP/HAM

Introduction

Infections with human T-lymphotropic viruses type 1 (HTLV-1) and type 2 (HTLV-2) are common in tropical regions of the world. They are acquired perinatally or through sexual contact or transfusion of blood products. HTLV-1 is transmitted to infants at birth by infected mothers during labor or delivery and through breastfeeding. Both types of HTLV can cause a variety of diseases, mainly lymphoproliferative disorders, myeloproliferative diseases, and neurological disorders. Infections of HTLV-1/2 are lifelong, with an asymptomatic carrier state.

The human T-lymphotropic viruses, type 1 (HTLV-1) and type 2 (HTLV-2), were the first human retroviruses discovered. They are single-stranded RNA retroviruses that encode for two glycoproteins, gp46 and gp21. They are classified as type C retroviruses, which are characterized by the presence of a pol gene that encodes for reverse transcriptase.

Infections with HTLV-1/2 are lifelong, with an asymptomatic carrier state. Over 20 million people are infected with HTLV-1/2 globally, with varying levels of prevalence in different regions. HTLV-1 is transmitted through blood or blood products, while HTLV-2 can be transmitted through sexual contact, transfusion of blood components, and perinatal transmission.

The first human T-lymphotropic viruses were discovered by Yves Gall at the National Cancer Institute in 1980 and 1982, respectively. They are single-stranded RNA retroviruses that encode for two glycoproteins, gp46 and gp21. They are classified as type C retroviruses, which are characterized by the presence of a pol gene that encodes for reverse transcriptase.

Conclusion: A prevalence of 5.1% of HTLV-1 among patients with lymphoid malignancies was found in this study. The patients were younger, more males, and HIV-negative.

Keywords: HTLV, Lymphoid malignancies, ATL, ELISA, TSP/HAM
seroprevalence reported in almost every region of the world. These retroviruses are found in foci of micro-endemicity, particularly in southern Japan, equatorial Africa, and parts of the Americas, including the Caribbean basin, and the Southeastern US. The frequency of antibodies in symptom-free adults throughout Sub-Saharan Africa has been reported to be from 3%-4%.\(^6\),\(^11\),\(^12\)

**Transmission**

Transmission of HTLV-1 occurs from mother to child, by sexual contact, blood transfusion, and by sharing contaminated needles. Mother-to-child transmission occurs primarily by breast-feeding through ingestion of infected milk-borne lymphocytes. In HTLV-1-endemic areas, approximately 25% of breast-fed infants born to HTLV-1-seropositive mothers acquire infection. The transmission efficiency is dependent on the duration of breast-feeding and the presence of maternal antibodies to HTLV-1. The time of infant seroconversion typically ranges from 1-3 years of age. Intrauterine or perinatal transmission of HTLV-1 does occur, but it appears to be less frequent than transmission by breast-feeding; approximately 5% of children born to infected mothers but not breast-fed acquire infection. Sexual transmission of HTLV-1 is bidirectional. However, the frequency of HTLV-1 transmission is much higher from male to female than from female to male. The presence of genital ulcers increases the risk of virus transmission.

Transmission of HTLV-1 by blood transfusion occurs with transfusion of cellular blood products (whole blood, red blood cells, and platelets) but not with the plasma fraction or plasma derivatives from HTLV-1-infected blood. Seroconversion rates of 44%-63% have been reported in recipients of HTLV-1-infected cellular components in HTLV-1 endemic areas. The probability of transmission by whole blood or packed red blood cells appears to diminish with greater duration of storage; this finding has been ascribed to a depletion of infected cells, presumably T-lymphocytes. Sharing blood-contaminated needles is the likely mode of transmission among intravenous drug users (IDUs).

Blood transfusion is a common occurrence amongst patients with lymphoid malignancies, whilst HTLV are transmitted through blood transfusion, screening for antibodies and discarding seropositive units should efficiently interrupt this transmission. Concern about HTLV-1 transmission through blood transfusion has led to the introduction of routine blood-donor screening for antibodies to HTLV-1 in developed countries. The decision to extend universal screening of blood donations to all industrialized countries with a low prevalence is a matter of debate and it has been suggested that the decision should be made country by country.

The study aims to determine the prevalence of HTLV-1 among patients with lymphoid malignancies and ascertain the relationship between HTLV-1 infection, and previous history of blood transfusion.

**Materials and methods**

**Patients**

A cross-sectional study was carried out at the haematology clinic and medical wards of the Lagos State University Teaching Hospital, between February and May 2012. Routine donor blood screening in Lagos does not include testing for HTLV, because Lagos is not considered an endemic area for HTLV. Ethical clearance for this study was obtained from the Health Research and Ethics Committee of Lagos State University Teaching Hospital.

Adult patients with lymphoid malignancies including Hodgkin lymphoma, non-Hodgkin lymphoma, chronic lymphocytic leukemia, acute lymphoblastic leukemia and multiple myeloma were included in this study. Hematology oncology patients with non-lymphoid malignancies were excluded from this study. Sample size was determined using the Yamane (Sloven) formula:

\[
n = \frac{N}{1 + N(e^2)}
\]

where n is the minimum sample size required, N is the population size; e is the degree of accuracy desired (ie, confidence interval) expressed as a decimal (±5% = ±0.05). Hematology department records of patients with lymphoid malignancies showed an average of 40 old and new patients were seen over a 3-month period. The minimum sample size can be calculated using N=40, and is shown below:

\[
n = \frac{40}{1 + 40(0.0025)} = \frac{40}{1 + 0.1} = \frac{40}{1.1} = 36
\]

obtaining a minimum sample size of 36 patients with lymphoid malignancies.

**Specimen collection and processing**

After obtaining consent, approximately 5 mL of venous blood was collected from each subject and placed into a sterile bottle. The blood was allowed to clot completely before centrifugation. The serum was separated within 6 hours and stored in sterile cryovials at -20°C.

Serum samples were assayed for HTLV-1 using the HTLV-1 and -2 Ab ULTRA version (Diagnostic Bioprobes
Patients (n) 4 (92.3%) were HBsAg positive, while 21 (53.8%) were males and 1 (53.8%) were females. The mean age of the patients was 51.9±17.8 years. The mean age of the male patients was 51.3±19.2 years, and of the female patients was 52.6±16.6 years. Of the 39 patients with lymphoid malignancies, six (15.4%) were single, 27 (69.2%) were married, one (2.6%) was divorced or separated, while five (12.8%) were widowed (Table 2).

Out of the 39 patients with lymphoid malignancies, 24 (61.5%) had solid malignancies while 15 (38.5%) had leukemias (Table 3). Of the 24 patients with solid malignancies, 18 (75%) had non-Hodgkin lymphoma, four (16.7%) had multiple myeloma, and two (8.3%) had Hodgkin lymphoma. Of the patients with leukemias, 13 (86.7%) had chronic lymphocytic leukemia, while (13.3%) had acute lymphoblastic leukemia.

Only two (5.1%) patients with lymphoid malignancies were reactive with an ELISA test. On confirmatory testing with Western blot, two (5.1%) patients with lymphoid malignancies were also positive for HTLV-1 (Table 4). One of the patients was a case of non-Hodgkin’s lymphoma, while the other was a case of chronic lymphocytic leukemia (Table 5).

Of the 24 patients with solid malignancies, one (4.2%) was positive for HTLV-1, while 23 (95.8%) were negative for HTLV-1. Of the 15 patients with leukemia, one (6.7%) was positive for HTLV-1, while 14 (93.3%) were positive for HTLV-1 (Table 5).

Among the 39 patients with lymphoid malignancies, 13 (33.3%) had history of previous blood transfusion, and 26 (66.7%) had no history of previous blood transfusion. Of the 13 patients with history of previous blood transfusion, none (0.0%) were positive for HTLV-1, while two (7.7%) of the 26 patients with no history of previous blood transfusion were HTLV-1 positive. Therefore there was no association between history of previous blood transfusion and positivity to HTLV-1 (P=0.544). Of the 13 patients with history of previous blood transfusion, two (15.4%) were HBsAg positive, while 11 (84.6%) were HBsAg negative. Among the 26 patients without history of previous blood transfusion, two (7.7%) were HBsAg positive, while 24 (92.3%) were HBsAg negative. There was no relationship between history of previous blood transfusion and HBsAg status (P=0.4073).

Table 1 Age and sex distribution of the subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Male</th>
<th>Female</th>
<th>Total (P=0.49)</th>
</tr>
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<tbody>
<tr>
<td>&lt;20</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20–29</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>30–39</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>40–49</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>50–59</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>60–69</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>70–79</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>80–89</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>90–99</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>21 (53.8%)</td>
<td>18 (46.2%)</td>
<td>39 (100%)</td>
</tr>
</tbody>
</table>

Table 2 Marital status of the subjects

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Patients (n)</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>6</td>
<td>15.4</td>
</tr>
<tr>
<td>Married</td>
<td>27</td>
<td>69.2</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Widowed</td>
<td>5</td>
<td>12.8</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Of the 13 patients with history of previous blood transfusion, one (7.7%) was positive for HCV, while 12 (92.3%) were negative for HCV. Among the 26 patients without history of previous blood transfusion, one (3.8%) was positive for HCV, while 25 (96.2%) were negative for HCV. There was no relationship between history of previous blood transfusion and HCV status (P=0.5614).

All 39 patients with lymphoid malignancies were HIV negative. Among the 15 patients with leukemia, two (13.3%) were positive for HBsAg, while 13 (86.7%) were negative for HBsAg. Of the 24 patients with solid malignancies, two (8.3%) were positive for HBsAg, while 22 (91.7%) were negative for HBsAg (Table 6). There was no difference in HBsAg status between the patients with leukemia and the patients with solid malignancies (P=0.5017).

Among the 15 patients with leukemia, one (6.7%) was positive for HCV, while 14 (93.3%) were negative for HCV. Of the 24 patients with solid malignancies, one (4.2%) was positive for HCV, while 23 (95.8%) were negative for HCV (Table 7). There was no difference in HCV status between the patients with leukemia and the patients with solid malignancies (P=0.6275).

Among the patients with lymphoid malignancies two (7.4%) of the 27 married patients were HTLV-1 positive. None of the single, divorced/separated or widowed patients were HTLV-1 positive. There was no association between marital status and HTLV-1 positivity (P=0.324).

Among the patients with lymphoid malignancies, two (5.1%) were HTLV-1 positive. One was aged 52 years old, and the other was 61 years old (P=1.000). Of the patients with lymphoid malignancies, two of the 21 males (9.5%) were positive for HTLV-1. No female was positive for HTLV-1. Sex differences were not statistically significant in this study (P=0.490).

## Discussion

A seroprevalence of 5.1% of HTLV was obtained among patients with lymphoid malignancies. This could be compared with 0.5% seroprevalence of HTLV in the general population in Lagos, who had no lymphoid malignancies. Based on these results, this study suggests a significant association between HTLV with lymphoid malignancies. The seroprevalence of HTLV obtained in this study is higher than that found among similar patients in Lagos by Analo et al in 1998, in which none of the patients with chronic lymphocytic leukemia and non-Hodgkin’s lymphoma were found to be HTLV-1 positive. However, it is lower than the seroprevalence of 18.8% reported by Rastin et al in Iran. Geographical differences and more sensitive methods (ie, polymerase chain reaction [PCR] and nested reverse transcription [RT]-PCR) used in the Iranian study could account for a higher prevalence obtained.

In 2004, Adedayo and Shehu found much higher sero-prevalence rates among patients with lymphoid malignancies in Dominica: 50% of Hodgkin lymphoma, 44.4% of non-Hodgkin lymphoma, and 37.5% of unclassified lymphomas were positive for HTLV-1.

History of previous blood transfusion appeared to have no impact on HTLV-1 status in this study as the two patients that were positive for HTLV-1 among the patients with lymphoid malignancies had no past history of previous blood transfusion. Furthermore, none of the 13 patients with history of previous blood transfusion was positive for HTLV-1. None of the...
blood donors had been previously transfused. Although it was not statistically significant, Murphy et al reported past history of transfusion as an important risk factor for HTLV-I seropositivity.\textsuperscript{32} The absence of positivity for HTLV-1 among the 13 patients that had been transfused in the past may be due to the fact that most transfusions in our center are administered with blood that had been stored in the refrigerator, which reduces the risk of transmission of HTLV-1 within days, due to death of HTLV-1 infected lymphocytes.\textsuperscript{34}

History of previous blood transfusion among patients with lymphoid malignancies did not appear to have any impact on infection with other transfusion transmissible viruses as all patients were HIV negative. Seropositivity to HBsAg and HCV was also not significant.

Several studies have demonstrated higher HTLV-1 prevalence with increasing age,\textsuperscript{35,36} in this study, seropositivity to HTLV-1 tended to occur in older age groups as both HTLV-1 positive patients were aged 52 and 61 years old, although the mean age of study population was 51.9±17.8 years.

Two patients who tested positively for HTLV-1 were males. This is not in conformity with studies that have shown the frequency of HTLV-1 transmission to be much higher from male-to-female than from female-to-male.\textsuperscript{22,23} This is most likely due to the higher proportion of males compared with females in this study. This is another limitation of the study, as the sex of the participants was skewed unintentionally in favor of the male sex. However, sex differences were not statistically significant in this study ($P=0.490$).

In conclusion, a prevalence of 5.1\% of HTLV was obtained among patients with lymphoid malignancies in this study, and a previous history of blood transfusion was not found to be a significant cause of HTLV-I infection.

**Disclosure**

The authors have no conflicts of interest in this work.

**References**


**Table 7** HTLV-I and HCV status of patients with lymphoid malignancies

<table>
<thead>
<tr>
<th></th>
<th>HCV positive</th>
<th>HCV negative</th>
<th>Total</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLV-I positive</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0.1012</td>
</tr>
<tr>
<td>HTLV-I negative</td>
<td>4</td>
<td>33</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>35</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** HCV, hepatitis C virus; HTLV, human T-lymphotropic virus.


