HUMAN T-CELL LEUKAEMIA VIRUS TYPE 1 (HTLV 1):
A PROSPECTIVE SERO-EPIDEMILOGICAL STUDY,
LUSAKA, ZAMBIA.

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KAS
1993

BY
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Presented to the University of Zambia in Partial
Fulfilment of the Master of Science degree in
Microbiology.

July 1993
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DECLARATION

I hereby declare that this dissertation is my own work and that it has not been previously submitted for Degree purposes here or at any other University.

........................................
Francis Chisaka Kasolo
LIST OF EXAMINEERS.
DEDICATION

This work is dedicated to my late uncle Fr. Pascal Mwamba Kakokota, who made my medical career, possible.
ACKNOWLEDGEMENTS.

I would like to thank Prof D. Morgan OBE for the advice, encouragement and supervision during my writing of this dissertation, my wife Edna for her tolerance and encouragement, my colleague and friend Dr Oshitani for his assistance, Dr H. Suzuki, for his help in counter confirming the positive samples, GRZ and JICA (Zambia) for the funding of the research.
SUMMARY

HTLV-1 is a retrovirus responsible for pathological disorders such as Tropical Spastic Paraparesis (TSP) and Adult T cell Leukaemia/Lymphoma (ATL). The main characteristic of HTLV-1 infection is its long period of latency and the age related sero-prevalence, especially in endemic areas. A sero-prevalence study of HTLV-1 at the University Teaching Hospital (UTH), Lusaka, Zambia, was done. Two populations namely healthy blood donors and patients admitted to UTH with pathology thought to be related to HTLV-1 infection were included in this study.

The objectives of this study were:

1. To establish the seroprevalence of HTLV-1 among blood donors and patients admitted to UTH.

2. To find out whether the presence of HTLV-1 specific antibodies has any relationship with age, sex, HIV infection, Hepatitis B infection or with any particular pathology.

Collected sera were tested for HTLV-1 specific antibodies by using Particle agglutination test (PA) and Enzyme immuno-sorbent assay (ELISA).

Serum positive on at least one test was subjected to a confirmatory Western Blot test, (Fijirebio inc).
A total of 339 blood donor and 107 patient sera were tested.

A total of 26 sera were positive on PA test, 2 were positive on ELISA, and none on Western Blot.

Of the sera testing positive on PA, 23 were from the blood donor sera and 3 were from patient sera.

On ELISA test one sample each from the blood donor and patient sera was positive. When the 26 PA positive sera were subjected to Western Blot test, 15 were clear negative, 11 were indeterminants and none were positive.

The commonest Protein band seen on Western Blot was the gag encoded protein P19. There was no significant relationship between HTLV-1 Indeterminants and HIV positive sera or between HTLV-1 indeterminants and Hepatitis B surface Antigen positive sera.

The test method used showed varied specificity and sensitivity. The difference may have been due to a false positive result, arising possibly from high
levels of circulating immune complexes commonly associated with frequent parasitic, bacterial and viral infections which are prevalent among populations in the tropics.

The absence of specific anti-HTLV-1 antibodies following the use of confirmatory test, may be related to sampling technique employed in this study.

On the other hand this could suggest a low prevalence of HTLV-1 infection in Zambia as opposed to reports from other African studies.

The indeterminant results obtained on Western Blot may support the long latency associated with HTLV-1 infection.

The indeterminants may be in the process of sero-converting and thus will need to be followed up over a period in order to establish their true status.

Cross reactivity with other retroviruses could also be a likely cause of indeterminant results.
INTRODUCTION:

Human T Cell lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) were the first human retroviruses to be discovered.(3). It is currently estimated that there are more than ten (10) million HTLV-1 carriers in the world. ATL and TSP/HAM are clinical conditions that are closely associated with HTLV-1 infection. (23)

In the Caribbean Islands and Japan, adult T-cell leukaemia (ATL) and tropical spastic paraparesis (TSP) or HTLV-1 associated myelopathies (HAM) are endemic. In these areas more than 5% of healthy individuals have antibodies to HTLV-1 (7).

Serological studies in Africa have shown that HTLV-1 is endemic in sub-Saharan Africa (4-6). Antibody prevalence in healthy African blood donors ranges from 0 to 9%. There have been reports of variation in sero-prevalence levels from several studies in Africa (4, 5, 6, 12, 13).

In a study carried out in Benin (West Africa), regional variation was observed between rural and urban areas. High rate of HTLV-1 infection was seen in rural areas as opposed to urban areas. High sero-prevalence rates of HTLV-1 correlated with high rates of disease (6).
Variation in data has also been linked to the type of test method used to detect HTLV-1. Until recently, techniques for HTLV-1 screening were not standardized. This contributed to variation in results even within the same population study. In Uganda, (R.4) one study showed a 0% prevalence rate while another (R.12) produced an 8% prevalence rate.

Substantial work has been done in countries of west central and north Africa towards the improvement of our knowledge on HTLV-1 infection, antibody prevalence, transmission and HTLV-1 associated disease in Africa. In Zambia, data on HTLV-1 is presently unavailable.

Lympho-reticular disorders/neoplasms, are not infrequent. Records from the UTH cancer registry and results from the histopathology department show that these disorders/neoplasms account for approximately 8% of adult malignancies seen in Zambia (32).

Some documented associated factors to HTLV-1 transmission like rural-urban migration, breast feeding and parasitic infections are prevalent in Zambia. It is thus possible that HTLV-1 is present in Zambia though not endemic.

The aim of this study is to find out the prevalence of HTLV-1 infection in blood donors and high risk groups in Zambia. The study further seeks to contribute to the literature on viral epidemiology in Zambia, especially HTLV-1 virology.
CHAPTER ONE

THE VIRUS

1. CLASSIFICATION AND MOLECULAR BIOLOGY

HTLV-1 is a type C retrovirus that can infect and transform mature T lymphocytes. The International Committee on the Taxonomy of Viruses (ICTV) has classified the HTLV-1 in the sub family Oncovirinae within the family Retroviridae (1).

HTLV-1 is a typical retrovirus by morphological appearance. It has a diameter of about 100nm, with an irregular envelope surrounding a symmetrical, central placed core with a dense nucleoid and a spherical shape.

It contains two copies of single-stranded RNA genome and is thus diploid. The RNA gene has 9 kilobase. The genome is coated with the nucleocapsid protein and the RNA-protein complex is enclosed in a capsid composed of multiple subunits (2).

Like all enveloped viruses, the envelope is acquired during budding from the host cell, except the surface (SU) and transmembrane (TM) glycoprotein which are virally encoded. Between the capsid and the envelope is a matrix (MA) protein.

The virion contains three virus-specific proteins that are essential for viral replication namely reverse transcriptase (RT), protease and an integrase (IN).
Like other retroviruses, HTLV-1 is highly tropic for 
CD4+ T lymphocyte.

HTLV-1 is structurally unstable after prolonged storage 
at -20°C and after exposure to heat (56°C), ether and 
acids. The Table below illustrates the classification 
on Human retroviruses:

Family: Retroviridae

Sub-Family: 

1. Oncovirinae  2. Lentivirinae  2. Spumavirinae
   Viruses     Viruses     Viruses: 
   HTLV-1      HLV-1      Formy Virus
   HTLV-2      HLV-2

2. EPIDEMIOLOGY OF HTLV-1

HTLV-1 was first described by Gallo and colleagues in 
1980 (R,3). Since then five more human retroviruses 
have been described. HTLV-1 has an epidemiological 
pattern of chronic endemic infection.

The distribution of the virus is sharply localised 
geographically.

There is a characteristic age-dependent sero-prevalence 
with a uniform excess in females. A recognized clinical 
outcome of chronic diseases of adults, with long latent 
period has been seen.

The natural history of HTLV-1 is poorly understood, 
since information on short to intermediate term 
outcomes is lacking.
PREVALENCE

Current data concerning HTLV-1 are limited primarily to cross-sectional and occasional sero-prevalence estimates.

No data is currently available to define the incidence of new infections in any population studied.

The general prevalence of infection in Africa is estimated to be less than 5% in a healthy population. In areas of high endemicity like Japan, the Caribbean and New Guinea, the positive rate is as high as 15% (4-7).

This reflects either the difficulties with the specificity of the assay or the low levels of the infection in a given environment.

SEX

There are more HTLV-1 antibody positive females than males. This predominance in sero-positivity, probably reflects the greater efficiency of sexual transmission from men to women (7-8).

ETHNICITY

A major determinant of HTLV-1 sero-positivity is ethnological. For example, on the cosmopolitan island of Trinidad, where persons of Asian and African ancestry are equally represented, HTLV-1
séro-positivity is virtually confined to the black population (9-10).
This racial or ethnic background may not be specifically related to susceptibility factors but reflects a pattern of transmission, particularly from mother to child.
Among the migrant population in Hawaii, sero-positivity is not related to race per se but reflects the tendency of the virus to cluster among persons with links to Japan. In Japan the general frequency of ATL is 2.5 cases per 1000 population in persons less than 40 years of age while in the carribean it is estimated at 2.8 cases per 100,000 population with all cases being of African ancestry (R.11).

EPIDEMIC BEHAVIOUR AND CONTAGIOUSNESS:
No epidemic of HTLV-1 infection has been documented anywhere in the world to date. All sero-positive individuals identified to date have had IgG antibody, suggesting chronic, long-term infection. Studies on transmission indicate that HTLV-1 is not easily transmissible and is less contagious than HBV or HIV (R.10).
AGE

HTLV-1 sero-prevalence has an age dependence in most geographical locations except in Africa (R.7, 13). Samples from different geographical regions have shown a low antibody carrier rate during childhood and adolescence; with a rise in sero-prevalence rate in adulthood (14, 15).

OCCUPATION

There is no relationship between sero-positivity and occupation especially in those traditionally at high risk of getting the infection e.g. Health workers (R.10).

TRANSMISSION

HTLV-1 is not readily transmissible virus and its transmission is closely cell-related. The major routes of transmission involve sexual spread, particularly from male to female (R.8), spread from mother to child, either perinatal or through breast milk, (R.16) and via blood transmission (R.17).

There is little evidence to support other modes of transmission such as via insects and the epidemiology pattern in population argue against any evidence for carnal transmission (R.10).

PATHOGENESIS: HTLV-1 AND ATL

The natural history of HTLV-1 infection and disease is
poorly understood. This maybe due to the very long latent period between exposure and disease outcome. Transmission studies suggest a 2 to 3 months incubation period and as long as several decades from initial infection to the onset of disease (R.10). Seroconversion is not associated with any acute symptoms. Molecular studies have documented the universal presence of the HTLV-1 virus in the genome of tumor cells (T-Lymphocytes), and this virus is always monoclonally integrated with a restriction enzyme pattern suggestive of random integration (R.2).

There is no single site for virus integration that acts as a trigger for leukaemogenesis. Considerable attention has now been focused on the possible role of the HTLV-1 viral transactivation gene and its potential for activating not only viral but also cellular gene(s) in the leukaemogenesis (R.2). Other data suggest that the virus may play an initiating role in oncogenesis but other co-factors may cause the progression to leukaemia (R.18).

Recently it has been suggested that a defect in the interleukin-2 (IL-2) receptor gene maybe connected to ATL. The virus elaborates a factor which activates the IL-2 receptor gene in an unregulated manner and the continuous expression of the IL-2 receptor contributes to the aggressiveness of the disease (R.33).
IMMUNITY AND VIRAL REPLICATION

Following infection with HTLV-1 both humoral and cellular responses occur. Sero-conversion occurs within 30-90 days following infection. Antibody levels remain stable for at least 1 year. These are predominately directed at the gag and envelope proteins.

In relation to clinical conditions, antibody titres are highest in TSP and lowest in ATL.

Clinically stable individuals have lowest antibody titres and these appear to disappear with age.

The presence of antibodies show a rapid or accelerated expression of viral antigen rather than a protective role (R.10).

Retroviral replication goes through the following stages as shown in figure below:
(I) Attachment of virus to target cell through the CD4 receptor.

(II) Uncoating and exposure of the viral genome.

(III) Reverse transcription (RNA to double stranded DNA).

(IV) Integration of viral DNA into host cell DNA (provirus stage).

(V) Transcription of viral DNA
(VI) Translation on viral mRNA

(VII) Assembly of virus and maturation

(VIII) Release of new viral particles.

CLINICAL FEATURE:

ADULT T CELL LEUKAEMIA/LYMPHOMA (ATL):

This neoplastic disorder was first reported by Takatsuki et al in 1976, in Japan.

Incubation period;

The incubation period for ATL is extremely long extending over several decades.

Characteristics:

ATL is characterized by acute mature T-cell lymphoproliferative malignancy with a rapidly progressive course. The major clinical features of this syndrome are: leukaemia, bone-marrow involvement, generalised lymphadenopathy, cutaneous involvement, hepatosplenomegaly and occasional lytic bone lesions. Patients present at an advanced stage of clinical disease and their mean age is 45 years with a range of 15-84 years.

The male to female ratio is equal.

Neurological syndromes (TSP):

These slowly progressive myelopathies affect primarily the sensory system.

Two major clinical subtypes fall under this syndrome, namely:
spastic and ataxic subtypes. These reflect the target nerve tracts that are affected. Clinical features include: paraparesis, impotence and difficulty with gait leading to total immobilization after a protracted period (R.20).

HTLV-1 associated meningitis has been reported (R.21). In Mozambique, Zaire and Tanzania a neurological entity known as Konzo has been reported. This condition is very similar to TSP but individuals with this condition appear not to carry HTLV-1 ANTIBODIES (R.22).

IMMUNOTYPE DISEASE:
HTLV-1 may be associated with an immune suppressive-like condition. Information to support this view is however absent (R.10).

DIAGNOSIS:
HTLV-1 associated disease is normally confirmed through laboratory diagnosis.

Methods currently available include:

1. **ANTIBODY DETECTION:**
   1. Particle Agglutination Test
   2. Enzyme Immune Assay Test
   3. Western Blot Technique

Most serological tests currently available are of poor sensitivity and cannot differentiate between HTLV-1 and HTLV-2. Future focus is on increasing the sensitivity and specificity of available assays..../11
2. **VIRAL ISOLATION**

Viral isolation as a means of diagnosis is not used routinely because it requires well established laboratories and a degree of sophistication (R.19).

3. **MOLECULAR METHODS**

Molecular virological methods are mainly reserved for use in research laboratories. These include **the polymerase chain reaction (PCR)** and molecular hybridization technique.

**PREVENTION/CONTROL**

Prevention and control of HTLV-1 infection may be achieved by:

1. Screening blood, especially in the endemic areas, for HTLV-1 antibodies, so as to prevent transfusion of infected blood.

2. Discouraging breast feeding in HTLV-1 positive mothers

3. Theoretically, by vaccination, although currently no vaccine is available. It may appear that what has been learned from the HIV-1 vaccine programme, such as injection of soluble gp160 may have a direct application to future efforts in HTLV-1 vaccine development (R.34)

**TREATMENT**

Combined chemotherapeutic approaches that are beneficial for some forms of aggressive T-cell lymphomas,
are only moderately effective in HTLV-1 associated leukaemia/lymphoma.

Experimental therapeutic use of monoclonal antibodies to leukaemic-associated cell surface protein has yielded no conclusive benefits.

Use of antivirals, such as deoxycoformycin, in the treatment of HTLV-1 associated T-cell leukaemia, has not been beneficial (R.23).
CHAPTER TWO

GENERAL OBJECTIVES

(a) To establish the HTLV-1 antibody prevalence among adult blood donors at the University Teaching Hospital, Lusaka, Zambia.

(b) To find out the HTLV-1 antibody prevalence in high risk group, i.e.

1. Frequent blood recipients
2. Patients with lympho-reticular disorders/
   Neoplasms and
3. Patients with neurological disease admitted to UTH.

SPECIFIC OBJECTIVES

1. To assess and find out the distribution of antibodies by:

   (a) age and sex
   (b) diagnosis

2. To determine the interaction of HTLV-1 with HIV-1 and HBV.
CHAPTER THREE

PATIENTS AND METHODS

STUDY SITE AND POPULATION

Samples for this study were obtained from the medical wards and Blood Bank Unit (BBU) of the University Teaching Hospital (UTH), Lusaka.

Lusaka is the capital city of the Republic of Zambia and has a population of about 1 million inhabitants. The UTH is the largest hospital in the country. It serves as a national referral hospital for patients from within and outside Lusaka. Sick adults who present at the UTH are first seen by a Medical or Clinical Officer in the Filter clinic (OPD).

A decision is made on whether a patient requires admission to hospital. Admitted persons are then referred to the admission ward and after that to the medical block. An average of 40 patients per day are admitted to the medical block.

Majority of these have Respiratory infections, malaria, or diarrhoeal disease. Less than 8% of total admissions have Lympho-reticular disorders.

The BBU currently operates as part of UTH and provides blood bank services for the hospital. An average of 8,000 blood units are collected per year for this purpose. Most of these units are obtained from voluntary donors like factory workers, college students,
and military recruits. Occasionally close relatives of admitted patients, donate blood for their sick relatives.

The present study covered a period of 4 months from January to April 1992. During this period approximately 10,000 patients were admitted to the medical wards of UTH and about 1,500 units of blood were collected by the BBU during this period.

**STUDY TYPE**

The present study was designed to be a descriptive longitudinal study.

**SAMPLING TECHNIQUE**

Due to time factor, the sample available then was used. This type of sampling may not typify a given population and may introduce bias in the study discussion. Ideally, random sampling should have been used.

**CALCULATION OF SAMPLE SIZE**

1. **ASSUMPTION FOR BLOOD BANK UNIT (BBU)**
   
   If we assume a BBU population of 1,500, an expected frequency of 2%, the worst acceptable result being 1%, and 95% confidence level, a sample size of 303 randomly selected donors would been required.

2. **ASSUMPTION FOR CLINICAL CASES**
   
   In this population, if we assume that there were
10,000 patients admitted during the study period, and an expected frequency of 4%, the worst result being 1% and 95% confidence level, a sample size of 91 randomly selected cases is necessary.

3. **STUDY POPULATION**:

   This study had two groups admitted:

   (a) **CLINICAL PATIENTS**

   Those studied were 18 years and above with any of the following diagnoses:

   1. Kaposi's sarcoma
   2. Undiagnosed neurological disorder
   3. Persistent generalised lymphadenopathy not associated with tuberculosis
   4. Unexplained hepatosplenomegaly
   5. Leukaemia/lymphoma.
   6. History of at least 3 transfusions

   (b) **BLOOD DONORS**

   Blood donors that come to the BBU during the study period were asked to participate. Both patients and blood donors gave written consent before being admitted to the study.

4. **DATA COLLECTION**

   A one(1) paged questionnaire was filled for every person admitted to the study. (see appendix)

   Information on sex, age, diagnosis and home address were recorded.
4. For persons recruited from the medical wards, associated symptoms and signs were broadly defined.

5. **LABORATORY PROCEDURE** (see appendix)

Venous blood was collected. After it had clotted, the serum was recovered and stored at -20°C until further analysis.

Sera were tested for IgG antibodies to HTLV-1 by particle agglutination test (appendix) and Enzyme Linked Immunosorbent Assay (ELISA) (appendix). Positive sera on at least one of these tests, were re-tested by IgM and IgG Western Blot test for confirmation (see appendix)

A Western Blot assay was considered positive when it showed at least four (4) protein bands (p19, p24, p28 and p53) on the IgG Blot.

**DATA ANALYSIS**

Data obtained was analysed and subjected to the Fisher Exact test.
CHAPTER FOUR

RESULTS

1. BLOOD DONORS

SEX AND AGE:

A total of 339 healthy blood donors (276 males and 63 females) were tested.

Their ages ranged from 18 years to 52 years with a mean age 26.24 years.

ANTIBODY PREVALENCE IN DONORS:

Of these 23/339 were positive on Particle Agglutination test (20 males and 3 females).

Only 1/339 serum was positive on ELISA. When the Particle Agglutination positive sera were subjected to Western Blot test, 11/23 were indeterminants, 12/23 were negative and none were positive (Table 1 and 3).
### TABLE 1

**HTLV-1 ANTIBODIES IN HEALTH BLOOD DONOR AND PATIENTS**

<table>
<thead>
<tr>
<th>SUBJECT STUDIED</th>
<th>NO. TESTED</th>
<th>NO. POSITIVE</th>
<th>PERCENTAGE</th>
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<tbody>
<tr>
<td></td>
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<td>PA ELISA W.B.</td>
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<tr>
<td>ADULT BLOOD DONOR PATIENTS</td>
<td>339</td>
<td>23 1 11(1)</td>
<td>7.0 0.3 3.2(1)</td>
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<tr>
<td>NEUROLOGICAL DISORDERS</td>
<td>12</td>
<td>2 1 -</td>
<td>5.0 3 -</td>
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<tr>
<td>LYMPHOPORAFRIC DISORDER (PGL)</td>
<td>33</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>SICKLE CELL DISEASE (MULTIPLE BLOOD TRANSFUSION)</td>
<td>7</td>
<td>- - -</td>
<td>- - -</td>
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<tr>
<td>KAPOSIS SARCOMA</td>
<td>23</td>
<td>1 - -</td>
<td>4.3</td>
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1 = INDETERMINANT

### TABLE 3

**WESTERN BLOT RESULTS (FUJIREBIO INC)**

<table>
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<th>SAMPLE</th>
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<th>IgM</th>
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<td></td>
<td>P15 P19 P24 P28 P33</td>
<td>P15 P19 P24 P28 P33</td>
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<td>POSITIVE CONTROL</td>
<td>+ ++ + + +</td>
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<td>- - - - -</td>
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</table>
17/23 of those found PA positive were below the age of 30 years.

It is difficult to make a definite statement on sex difference as the female population studied was small (63/339) due to fewer female blood donors.

RELATIONSHIP WITH HBV AND HIV-1:

7/23 PA positive sera were HIV positive. 5/11 WB indeterminants were HIV positive (p = 0.1) (Table 4)

**TABLE 4**

CORRELATION THLV-1 (WB) INDETERMINANT AND HIV-1 POSITIVE AT UTH, LUSAKA, ZAMBIA

<table>
<thead>
<tr>
<th>HTLV-1</th>
<th>HIV</th>
<th>TOTAL</th>
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<tr>
<td></td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>P</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8</td>
<td>18</td>
</tr>
</tbody>
</table>

On the other hand only 2/23 of those PA positive were HBsAg positive (p=0.4 Not significant).

There was no significant relationship between
HBsAg positives and HTLV-1 WB indeterminants (p=0.1).

7/23 sera positive on PA had titres of more than 1:16 (Table 2)

**TABLE 2**

<table>
<thead>
<tr>
<th>NO. SAMPLES</th>
<th>TITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td><strong>TOTAL: 26</strong></td>
<td></td>
</tr>
</tbody>
</table>

2. PATIENTS

**AGE AND SEX**

A total of 107 patients (48 females and 59 males) with the stated clinical conditions were tested. The age range was 18 to 67 years with mean of 28 years.
ANTIBODY PREVALENCE IN PATIENTS

Patients were grouped as follows:

(a) NEUROLOGICAL PATIENTS

42/107 had unexplained neurological disease. 2 were positive on PA test, one (1) on ELISA and PA and none on WB (Table 1).

<table>
<thead>
<tr>
<th>SUBJECT STUDIED</th>
<th>NO. TESTED</th>
<th>NO. POSITIVE</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PA</td>
<td>ELISA</td>
</tr>
<tr>
<td>ADULT BLOOD DONOR PATIENTS</td>
<td>339</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>NEUROLOGICAL DISORDERS</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>LYMPHOPROLIFERATIVE DISORDER (PGL)</td>
<td>33</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SICKLE CELL DISEASE (MULTIPLE BLOOD TRANSFUSION)</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KAPOSI'S SARCOMA</td>
<td>23</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

1 = INDETERMINANT

12/42 (including the two patients who were PA positive) were HIV-1 positive.

(b) KAPOSI'S SARCOMA PATIENTS

23/107 of the patients had KS. 1/23 was PA positive but ELISA and WB negative.

There was a significant relationship between KS and HIV infection (15/23, p=0.04).
(c) **PGL**

33/107 patients had non tuberculous PGL. All were negative for HTLV-1 antibodies.

7/23 were HIV positive. None were HBsAg positive.

(d) **PATIENTS WITH RECURRENT TRANSFUSION**

7/107 patients had a history of at least 3 transfusions. All were patients of sickle cell disease and were negative for HTLV-1 HBsAg and HIV infection.
CHAPTER FIVE

DISCUSSION

Data from two (2) populations at UTH, Lusaka has been presented. This is the first sero-survey of HTLV-1 antibody to be done in Zambia.

The prevalence of HTLV-1 antibody in similar population in Africa varies widely from country to country (R4, 5, 6, 20, 21). In the above studies, the type of test method employed and age of sera used, may contribute to false positive result.

In the current study only recently collected sera was used. The results are therefore representative of the present HTLV-1 infection rate in those studied.

Three test methods were used each with different specificity, sensitivity and test principle.

The order of specificity and sensitivity starting with the test with lowest specificity and sensitivity is as follows: particle agglutination, ELISA and western blot respectively.

Among the apparently health blood donors sero-positive results were obtained in 23/339 (7.0%) by PA as opposed to only 1/339 and 0/339 by the more specific ELISA and WB respectively. This finding is similar to a study done in Johannesburg (22) and that from Nigeria (23).
False positive PA and ELISA result among Africans has been associated with high serum levels of immune complexes resulting from frequent bacterial, parasitic and viral infections (20).

The finding in this study reflects two possibilities, (a) the sampling technique used, (b) possibly a very low HTLV-1 prevalence rate of less than 1%, as opposed to Japan (14), the Caribbean (11, 12) and other parts of Africa (5,6,20,22,23). Age as a factor to the results obtained is debatable. Work on HTLV-1 and age distribution has given conflicting results with some (5, 6, 14), showing an age dependent results, with those above 40 years having the highest positive rates, while other reports (20, 22,23) do not support this finding.

A notable finding in this study was the frequency of indeterminant reactions on Western Blot. Confirmatory WB can avoid non specificity in the sense of excluding false positive reactions (e.g. reactivity with contaminants of debris or general elevation of serum immunoglobulins level). The prevalence of true positive sera would tend to be under estimated in circumstances of high non specific background. Indeterminant reactions have been observed in work among Africans (24).
Indeterminant results on WB may also support the long latency associated with HTLV-1 infection. These may be in the process of sero-converting and need to be followed up over a period in order to establish their true status.

In this study indeterminants results did not correlate significantly (p=0.1) with HIV antibody positivity. The possibility of cross reactivity with other Retroviruses like HTLV-2 was however not ruled out. The dominant protein bands were the p15 and p19. These findings imply that, the commercial kits currently available need to be evaluated for specificity so that only HTLV-1 positive serum is detected.

The fact that all patients with lympho-proliferative disorders (PGL and Kaposi's sarcoma) were sero-negative on confirmatory test is not surprising. Sero-positivity has rarely been reported among African patients with other forms of lympho-proliferative disorders apart from ATL, which is very rare in tropical Africa (22).

Patients with neurological disorder were not confirmed HTLV-1 positive. Studies done on neurological patients in Central Africa have shown that HTLV-1 plays a limited pathogenic role and suggest that development of neuro myelopathies in Africa may not be related to HTLV-1 (22,26,25).
The above findings also support the clinical observation that tropical spastic paraparesis is extremely rare in Zambia (32). Both patients with sickle cell disease who had had multiple blood transfusions were sero-negative. No specific conclusion would be drawn from this because the number studied was too small.
CONCLUSION

1. HTLV-1 antibody were not confirmed in sera tested. However if we relate this to the sample size then HTLV-1 may occur at less than 1% prevalence in Zambia.

2. There was no difference in results obtained from clinical cases and blood donors.

3. Sera that showed indeterminants results on WB test did not relate to HIV-1 positivity significantly.

4. Test methods used gave varied results, with PA test giving the highest false positive results.
RECOMMENDATIONS

1. Larger prospective studies are now necessary in Zambia to clarify the extent of HTLV-1 in the community.

2. There is need to phenotypically classify Lymphoproliferative malignancies and also look at the percentage of the population infected with HTLV-1 who eventually develop ATL.

3. Age as factor in HTLV-1 positivity should be investigated.
### APPENDICES I

### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immuno-Deficiency Syndrome</td>
</tr>
<tr>
<td>ds-RNA</td>
<td>Double stranded - Ribonucleic acid</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immunosorbent-assay</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immune Deficiency Virus</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>Human T cell Lymphotrophic Virus type 1</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>PA</td>
<td>Particle agglutination test</td>
</tr>
<tr>
<td>WB</td>
<td>Western Blot</td>
</tr>
<tr>
<td>BBU</td>
<td>Blood Bank Unit</td>
</tr>
<tr>
<td>ATL</td>
<td>Adult T cell Leukaemia/ Lymphoma</td>
</tr>
<tr>
<td>HAM</td>
<td>HTLV-1 related myelopathies</td>
</tr>
<tr>
<td>TSP</td>
<td>Tropical Spastic Paresis (same as HAM)</td>
</tr>
<tr>
<td>UTH</td>
<td>University Teaching Hospital</td>
</tr>
</tbody>
</table>
APPENDICES II

1. QUESTIONNAIRE FOR HTLV-1 SERO-EPIDEMIOLGY RESEARCH

SERIAL NUMBER ....................
BLOOD BANK/NOSP No. ................
AGE: ............... SEX ...........
AREA OF RESIDENCE: ..............
DIAGNOSIS IF ANY: ..................
ASSOCIATED SYMPTOMS: ..............
......................................
......................................

LABORATORY RESULTS

HTLV-1:
1. PARTICLE AGGLUTINATION:    P/N
2. ELISA:    P/N
3. WESTERN BLOT:
  IgG:  P15::   P19::   P24::   P28::   P53::   
  IgG:  P15::   P19::   P24::   P28::   P53::   

INTERPRETATION:

HIV1+2:
WELCOZYME:    P/N.

HEPATITIS B SURFACE ANTIGEN:

SERODIA HBS:    P/N

SIGNATURE: ___________________________

DATE:   _______ / _______ / _______
APPENDICES III

CONSENT FORM

I ____________________ fully understand the nature of this study and has no objection in participating in the study.

I further know that the results of this test shall be kept confidential and that the samples collected may be used for any additional test that may be required.

Name: ____________________  Sign: ____________

Witness: ____________________  Date: ____________

Doctor's Name: ________________
SERODIA PHA TEST FOR HTLV-1:

PRINCIPLE:

(THE SAME AS THAT OF HBsAg)

TEST PROCEDURE:

1. Put 25ul of diluent into well 1-3
2. Put 25ul of serum sample in the first well and dilute serially up to row 3
4. Mix and incubate at room temperature for 2 hours.

INTERPRETATION:

![Image of test results]

- NEG
- POS
APPENDICES V

WESTERN BLOT TECHNIQUE (FUJIREBIO INC):

PRINCIPLE:

Specific HTLV-1 proteins are separated according to molecular weight by gel electrophoresis in the presence of SDS. The fractionated proteins are transferred by electroblotting onto a sheet of nitrocellulose which is then washed and cut into strips.

TEST PROCEDURE:

To do the test, wash/diluent and the patients' serum are incubated with the strip; the HTLV-1 antibody present in the serum will bind to viral antigens located on the strip as discrete bands.

Unbound material is discarded, and the strip is washed and then substrate solution is added and then incubated. The substrate solution is discarded following incubation and the strip is washed again. The procedure stops the enzyme reaction. If antibodies to the major HTLV-1 antigens are present in the serum in sufficient concentration, bands corresponding to the position of one or more of the following proteins will be seen on the nitrocellulose strip; P15, P19, P24 and P53.

(The same protein bands are used for IgG and IgM)

(SEE DIAGRAM NEXT PAGE)
APPENDICES VII

HIV WELLCOzyme ELISA 1+2

PRINCIPLE OF THE TEST:

The Wellcozyme 1+2 test is based on highly purified immunodominant antigens of the core and envelope proteins of HIV-1 (Weiss isolate) from cultures of bacteria modified by molecular biological techniques; and an immunodominant epitope of the HIV-2 envelope which is modified to ensure specificity and sensitivity. These antigens are immobilised onto micro wells. The conjugate is a mixture of the same antigens which have been labelled with the enzyme alkaline phosphatase.

Test samples and control sera are incubated in the wells and antibodies to HIV-1 or to HIV-2 present in the samples bind to the antigens on the microwell.

In a subsequent step, conjugate is added which in turn binds to any specific antibody already bound to the antigen on the well. Samples not containing specific antibody will not cause the conjugate to bind to the well.

In the third and fourth steps any conjugate bound to the well is detected by the addition of an amplifying substrate system for alkaline phosphatase. After incubation the enzymic actions are stopped with sulfuric acid and the colour is read spectrophotometrically at 492nM.
The amount of conjugate and therefore colour, in the well is directly related to the concentration of antibody to HIV in the sample.

TEST PROCEDURE:

1. Add 25ul of test sample to each well, leaving 6 wells for control sera.
2. Add 25ul of the negative control to 4 wells.
3. Add 25ul of the positive control to the remaining 2 wells.
4. Cover wells with a lid and incubate at 37°C for 30 minutes.
5. At the end of incubation was the wells.
6. Add 50ul of conjugate solution to each well immediately after wash is completed.
7. Incubate at 37°C for 30 minutes under humid condition.
8. Wash plate again.
10. Incubate for 20 minutes under humid conditions.
11. Add 100ul of amplifier solution and incubate for 10 minutes at 20-25°C. Keep away from direct light.
12. Add 50ul of stop solution.
13. Read the absorbance of each well at 492nm.
INTERPRETATION:

Obtain mean absorbance of negative controls. Cut off value is obtained by adding 0.200 to the mean of negative control.

Absorbance less than the cut off is negative while that which is above cut off is positive.
APPENDICES VII

HEPATITIS B SURFACE ANTIGEN: (SERODIA PHA)

PRINCIPLE OF TEST:

This test is based on the principle that sensitized red blood cells consisting of fixed chicken erythrocytes with absorbed, highly purified guinea pig anti-HBs immunoglobulin (IgG), are agglutinated specifically in the presence of HBs Antigen in the serum (Plasma).

TEST PROCEDURE:

1. Prepare serum by centrifugation.
2. Do the test in 'U' shaped microplates.
3. Dispense 50ul and 25ul of serum diluent into the first row and second row respectively.
4. Add 5 ul of the test serum to the first row.
5. Make serial dilution from first row to second row using a 25 ul diluters.
6. Add 25ul of control cells to the first row and 25ul of sensitized cells to the second row.
7. Mix and incubate at room temperature for 1 hour and then read the results.

INTERPRETATION:

(SEE DIAGRAM BELOW)
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