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Research Article

HIV and HTLV Co-Infection in Abang Minko'o Area of the South Region of Cameroon

Fonweban Yvonne Ngoikuh[1]*, Dora Mbanya[2] and Claude Tayou Tagny[3]

[1]*General Medicine, Faculty of Medicine and Biomedical Sciences Yaounde, Cameroon*

[2]*Hematology, Faculty of Health Sciences Bamenda, Cameroon*

[3]*Hematology, Faculty of Medicine and Biomedical Sciences Yaounde, Cameroon*

***Corresponding Author:** Fonweban Yvonne Ngoikuh, General Medicine, Faculty of Medicine and Biomedical Sciences Yaounde, Cameroon.

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Abstract

Introduction: The human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV) have gained global concern in the last decades. Infection with HIV is a major public health problem worldwide responsible for diverse clinical manifestations with increased morbidity and mortality in infected individuals. The emergence of several pathogens like HTLV in HIV carriers has complicated the management of the coinfection as there is neither a cure nor effective treatment for HTLV infection. The impact of co-infection of these two viruses on the immune system and disease progression remains unclarified. These retroviruses share similar routes of transmission and as a result co-infection is not rare in areas where they both co-exist. Cameroon which is an endemic zone for HTLV, has one of the highest HIV prevalence rates in Central Africa. However, information on HIV and HTLV co-infection in Cameroon is sparse. As a result, we carried out this study in a border area of the South Region of Cameroon.

Objectives: This study was carried out with the main aim to determine the prevalence of HIV and HTLV co-infection in the Abang Minko'o area of the South Region of Cameroon.

Methods: From October 2014 to May 2015, we carried out a cross-sectional analytic and descriptive study, in which pre-structured questionnaires were completed by the principal investigator and blood samples were collected from adults in the Abang Minko'o area of the South Region of Cameroon. Abang Minko'o is a border area located at about 220 km from the capital city, Yaounde and 1.5 km away from the border between Cameroon and Gabon. It is a commercial area with an international market, 'Marché Mondial' which attracts people from different regions in Cameroon, Gabon, Equatorial Guinea, Sao Tome and Principe, and Congo, and who all interact freely there. Hence, sexual promiscuity is high in this area. The blood specimens collected were screened for HIV using two rapid tests, Determine® HIV 1/2 test and ImmunoComb® II HIV 1 and 2 BiSpot test on the field site in Abang Minko'o, and the samples were transported to Yaounde, where in the Haematology Laboratory of the Yaounde University Teaching Hospital, further screened using an Enzyme-Linked ImmunoSorbent assay, Murex HIV Ag/Ab Combo. The samples were also screened for HTLV using the rapid test ImmunoComb® II HTLV 1 and 2 assay, which does not distinguish between the two types. Data was entered using Microsoft Excel 2013 and analyzed using SPSS version 20.0. Where appropriate, the Chi Square test was used to establish risk ratio (RR), confidence intervals (CI) and p-values. All p-values less than 0.05 were considered statistically significant.

Results: Out of 157 participants' blood specimens analyzed, 10 were seropositive for HIV infection giving a prevalence rate of 6.4%. The prevalence was highest in the age group of 50-59 years (25% that is 3 participants out of 12) followed by in young adults of 30-39 years (9.5% that is 4 participants out of 42). Females were more infected (7.1%) than males (5.7%). The natives from Abang Minko'o called the Ntoumous, had the highest HIV prevalence rate (8.4%). Infection with HTLV 1 and 2 was found in 35 out of the 157 participants included in our study, giving a prevalence rate of 22.3%. The prevalence of HTLV generally increased with age and was highest amongst the age group 50-59 years (41.7%). Males were more infected (26.4%) than females (17.1%) with a sex ratio of 1.92.Co-infection with HIV and HTLV 1 and 2 was found in 4 out of the 157 participants, giving a prevalence rate of co-infection of 2.5%. The prevalence of HTLV 1 and 2 in HIV-1 positive participants was 40% which was 16 times more when compared to the co-infection rate in the general population. All the co-infected participants were natives from Abang Minko'o, the Ntoumous with males more infected than females (sex ratio 3:1). The trends of HIV and HTLV 1 and 2 infections in seropositive participants for each virus were almost similar.

Amongst the risk factors associated with sexual behaviour and HIV transmission identified, sex-money exchange (prostitution) and a history of sexually transmitted infections (STIs) were significantly associated with HIV infection. Those practising sex-money exchange were more infected (10.5%; p = 0.039) and almost 2 times more at risk of HIV infection (RR 1.729; p = 0.039), and those who did so regularly were 4 times more exposed (RR 4.450; p = 0.075) even though this difference was not statistically significant. Participants with a history of STIs were more infected (12%; p = 0.048) and were 2 times more exposed to HIV infection (RR 2.005; p = 0.048). However, some other risk factors identified though not statistically significant, exposed more to HIV infection like alcohol

consumption (RR 1.097; p = 0.786), cigarette consumption (RR 1.838; p = 0.381), visitation of night clubs or bars (RR 1.114; p = 0.754) with 2 times more exposure in regular visitors (RR 2.066; p = 0.506), sexual activity (RR 1.035; p = 0.553), age at first sex <16 years (RR 1.216; p = 0.260), having more than one sexual partner in the last 12 months (RR 1.421; p = 0.206), cheating on partner (RR 1.452; p = 0.124), homosexuality (RR 3.675; p = 0.205) and having a history of induced abortion or unwanted pregnancy (RR 1.405; p = 0.313). The use of condoms was found to be protective against HIV infection (RR 0.808; p = 0.455) and consistent use was even more protective (RR 0.000; p = 0.554) than inconsistent use (RR 0.854; p = 0.554).

Conclusion: We can conclude that the prevalence of HIV and HTLV 1 and 2 are high in Abang Minko'o. Co-infection of HIV and HTLV 1 and 2 is not rare. The prevalence of HTLV 1 and 2 in HIV-1 infection is high. Sexual behaviours are less tempered by social norms and values in this border area. The Abang Minko'o population constitutes a potentially vulnerable group for HIV and HTLV transmission. We can conclude that the prevalence of HIV and HTLV 1 and 2 are high in Abang Minko'o. Co-infection of HIV and HTLV 1 and 2 is not rare. The prevalence of HTLV 1 and 2 in HIV-1 infection is high. Sexual behaviours are less tempered by social norms and values in this border area. The Abang Minko'o population constitutes a potentially vulnerable group for HIV and HTLV transmission.

Recommendations: The Ministry of Public Health should emphasize on the need for regular and consistent use of condoms especially in mobile populations as a tool to prevent HIV transmission and co-infections, and also evaluate the cost-effectiveness of systematic screening for HTLV 1 and 2 on all blood donors before transfusion of collected samples in order to prevent transmission of the virus by blood transfusion through complementary studies on HTLV nationwide.

Keywords: Retroviruses; HIV; HTLV; Co-Infection; Prevalence; Risk Factors; Sexual Behavior; Border Area

Abbreviations

%: Percentage; µL: Microlitre; AB: Antibody; Ag: Antigen; AIDS: Acquired Immunodeficiency Syndrome; AP: Alkaline Phosphatase; CD: Cluster Of Differentiation; CI: Confidence Interval; CNS: Central Nervous System; DHS: Demographic Health Survey; EDTA: Ethylene Diamine Tetra Acetate; EIA: Enzyme Immunoassay; ELISA: Enzyme-Linked Immunosorbent Assay; FMBS: Faculty Of Medicine And Biomedical Sciences; GB: Gigabytes; HIV: Human Immunodefiency; Syndrome; HTLV: Human T-Lymphotropic Virus; RR: Risk Ratio; STI: Sexually Transmitted Infection; STLV: Simian T-Lymphotrophic Virus; TMB: Tetramethylbenzidine; USB: Universal Serial Bus; WHO: World Health Organisation; YUTH: Yaounde University Teaching Hospital

Introduction

The acquired immunodeficiency syndrome (AIDS) is a pandemic disease and since its discovery in 1981 and its etiologic agent, the human immunodeficiency virus (HIV) in 1983, it is still a major public health problem. According to statistics from the World Health Organisation in 2013, 35 million people were living with HIV worldwide, 2.1 million were newly infected that year and 1.5 million AIDS deaths were registered [1]. The WHO estimates in 2012 showed that Sub-Saharan Africa still has the greatest prevalence with 4.7% of adults living with HIV infection [2]. Cameroon's prevalence rate was estimated at 4.5% [2]. Despite the low infection rate among the general population, there is a broad genetic diversity of the viruses infecting individuals in Cameroon, with numerous divergent subtypes, sub-subtypes and recombinant forms of HIV-1 group M viruses [3-8]. With new recombinant forms arising continuously in different regions of Cameroon and globally, the genetic diversity of HIV-1 virus poses a major challenge for diagnosis, treatment, and vaccine development [3,4,9,10].

The Human T-lymphotrophic virus (HTLV), like HIV, is a retrovirus known to have four types: 1, 2, 3 and 4. [11]. The HTLV-1 infection has been found in 10 to 20 million people worldwide [12] and has been diagnosed on all the five continents since it was first described in 1980 [11]. The HTLV is particularly endemic in Japan, Melanesia, Africa and South America [13]. The four HTLV types are suspected to have originated from cross-species transmission of simian T-lymphotropic viruses (STLVs) to humans, though an STLV equivalent of HTLV-4 is yet to be identified [14]. The HTLV type 1 and 2 are known to infect people globally and cause disease in 5% of the population but very little is known concerning the HTLV type 3 and 4 epidemiology and public health significance. Thus, further studies are necessary to determine the prevalence, geographic distribution, and disease potential of these emerging retroviruses. The HTLV type 3 and 4 were first discovered in Cameroon in 2005 [14]. Furthermore, cases of HTLV-3 infection in persons living in Cameroon have been reported suggesting that this virus is not extremely rare in the human population living in Central Africa [15]. A prevalence study carried out by Zheng., *et al*. in 2010 showed a prevalence rate of 7.2% for HTLV type 3 in the rural population of Cameroon [14]. Another study carried out in the South of Cameroon by Filippone., et al. in 2012, showed a prevalence rate of 1.93% for HTLV type 1, 0.66% for HTLV type 2 and 65.65% of the samples were Western blot sero-indeterminate [16].

The HIV and HTLV have similar modes of transmission through blood products, sexual contact especially heterosexual, vertical transmission from mother to child and through breast milk [17]. They also have the same target cells, CD4+ and CD8+ T lymphocytes [17]. The impact of their co-infection is still very much unclear in most parts of the world [18]. Their co-infection rate is variable worldwide and the highest prevalence has been noted in

large urban areas in the Americas, Europe, and Africa [19]. In a study carried out by Ndumbe., *et al.* in 1993 among pygmies in the East Region of Cameroon, HTLV-1 was found in 10.9% and HIV in 0.7% of the study population [20]. A study on serologic findings in blood donors in Cameroon revealed a prevalence of 7.9% of HIV and 1.6% of HTLV-1 infection among first-time donors in Yaounde [21]. These two retroviruses share the same risk factors (unprotected sexual intercourse, intravenous drug use, prolonged breast feeding, history of blood transfusion, prostitution) and the same mode of transmission. This is why co-infection between HIV and HTLV is not rare in endemic zones like South Western Japan, the Caribbean Basin, South America, parts of the Middle East, Melanesia, the West Indies, Jamaica, and Central Africa [22].

Even though these two viruses have different biologic characteristics and clinical expressions, they have the same tropism for CD4+ T lymphocyte cells. The HIV has been shown to increase the viral load of HTLV type 1 [23] and that inflammatory complications were more frequent in co-infected individuals especially on antiretroviral therapy [24-26]. However, in-vitro studies have shown that HTLV type 1 provokes an activation of HIV [27] through diverse cytokine reactions. In clinical studies, an increase in CD4 count has been frequently noted in co-infected persons with no corresponding immunologic benefit [28] as HTLV type 1 stimulates the proliferation of CD4+ T lymphocytes but affects their proper functioning. It seems co-infection with HTLV type 1 worsens and/or accelerates HIV infection [29] and increases mortality by AIDS [30,31] but there are discordant results [30,33,34] preventing the drawing of a definite conclusion.

Thus, the South Region of Cameroon which was reported to have the highest prevalence of HIV infection in the national territory in 2011, is also an endemic zone for HTLV infection. Hence the reason for this study.

Materials and Methods

Materials

Materials used for sample collection, storage and transportation

- EDTA tubes 5ml
- Insulated boxes
- Latex gloves
- Local transportation cars
- Tourniquet
- Vacutainer adaptors
- Vacutainer needles.

Materials for analysis and write ups

- Research questionnaires (socio-demographic, risk factors associated to sexual behaviour and HIV infection, HIV and HTLV serologic data)
- Registers
- Writing materials (Pens, pencil, correcting ink and eraser).
- A4papers
- A computer with Microsoft Word, Microsoft excel and SPSS program.
- A USB flash disk of 4GB.

Methods

In order to attain the different specific objectives the following procedure were used; invitation and selection of participants, pre-counselling and blood samples collection, sample management and storage, sample screening tests, post-counselling and delivery of results, data analysis.

Participant invitation and selection

The local Health Centre served as one point for the sensitization about HIV screening. In addition, the community was also sensitized through announcements in the various churches and schools. Posters with information on HIV screening were also pasted on strategic areas in the market as well as on mobile trucks, buckets of mobile vendors, cars and buses. Potential participants were asked to report at the Abang Minko'o market controller's office, where all who met the study criteria gave their informed consent. Pre-test counselling was given to all consenting persons individually or as a group by the principal investigator, and a pre-structured questionnaire was completed by the principal investigator since the participants came in gradually and did not have to line up. Post-test counselling was done by the principal investigator who was trained to do so during Integrated Health posting, individually after HIV testing. All HIV infected participants were referred to the Ambam District Hospital for further management.

Sample collection

For each recruited participant, 5ml of blood was collected into Ethylene Diamine Tetra Acetate (EDTA) containing tubes by venipuncture using standard techniques and materials (gloves, prepared sterile alcohol swabs, tourniquet, vacutainer adaptors and vacutainer needles), respecting aseptic rules with the help of a laboratory technician. Each sample was coded with an identification number.

Sample management and storage

Due to lack of electricity at the field site in Abang Minko'o, we did not have the possibility to use a centrifuge. The blood samples collected were kept for a few hours for plasma to separate from cellular blood components. The samples were then managed as shown on figure 1 below.

- Part of the separated blood plasma collected was used immediately to screen serially for HIV using two rapid tests.
- HIV was screened using the rapid screening HIV test with DetermineTM HIV1/2 (Alere; Bedfordview, South Africa).
- Samples that tested positive for HIV with Determine were further tested using ImmunoComb® II HIV 1 and 2 BiSpot before the results were given to the participants.
- The rest of the samples in the EDTA tubes for all participants were later transported using insulated boxes with ice cubes to the Haematology Laboratory of the YUTH for further tests.

- Confirmatory tests for HIV were done using enzyme-linked immunosorbent assay (ELISA): Murex HIV Ag/Ab Comboa (DiaSorin; Saluggia, Italy).
- The blood samples were also screened for HTLV using the ImmunoComb® II HTLV 1 and 2 assay.

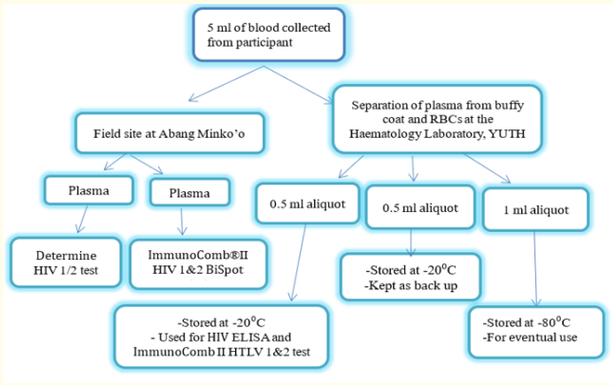

Figure 1: Sample management and storage at the different study sites.

Sample Analysis

Serologic testing for HIV and HTLV infection was done on all the samples collected in the following locations:

HIV

Field site in Abang Minko'o

- **Rapid tests:** DetermineTM HIV1/2a (Alere; Bedfordview, South Africa) and Immunocomb® II HIV 1 and 2 BiSpot.

Haematology Laboratory of the YUTH

- **Confirmatory test:** ELISA; Murex HIV Ag/Ab Comboa (DiaSorin; Saluggia, Italy).

HTLV

Haematology Laboratory of the YUTH

- **Rapid test:** ImmunoComb® II HTLV 1 and 2 (Orgenics; Yavne, Israel).

HIV Screening

The Determine™ HIV1/2 (Alere; Bedfordview, South Africa) rapid test was initially performed on all samples. Samples with positive results were further tested using the ImmunoComb® II HIV 1 and 2 BiSpot test. All samples with positive and negative results were further analysed using Enzyme-linked immunosorbent assays (ELISA), the MUREX (Murex Biotech Limited, Dartford, United Kingdom) combination antigen/antibody test.

HIV screening with determine™ HIV1/2

Principle of determine™ HIV1/2

Determine™ HIV1/2 is an in vitro, visually read, qualitative immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood from infected individuals. In this, the test sample was added to the sample pad. As the sample migrated through the conjugate pad, it reconstituted and mixed with the selenium colloid-antigen conjugate. This mixture continued to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. Any antibodies to HIV-1 and/or HIV -2 present in the sample bound to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. In the absence of antibodies to HIV-1 and HIV-2, the antigen-selenium colloid flowed past the patient window and no red line was formed at the patient window site. To ensure the assay's validity, a procedural control bar always is incorporated in the assay device.

Test procedure

1. The protective foil cover was removed from each test.
2. 50 µL of plasma sample was applied to the sample pad using a precision pipette.
3. The results were read after a minimum of 15 minutes.

Interpretation of results (Figure 2)

- **Positive (Two Bars):** The result was positive when the red bars appeared in both the control window (labelled "Control") and the patient window (labelled "Patient") of the strip. Any visible red colour in the patient window was interpreted as positive.
- **Negative (One Bar):** The result was negative when one red bar appeared in the control window of the strip (labelled "Control"), and no red bar appeared in the patient window of the strip (labelled "Patient").
- **Invalid (No Bar):** When there was no red bar in the control window of the strip, even if a red bar appeared in the patient window of the strip, the result was invalid and was repeated.

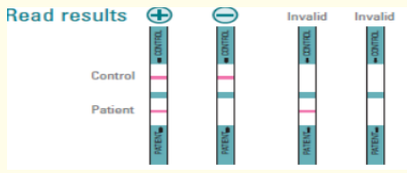

Figure 2: Interpretation of DetermineTM HIV1/2 results (109).

The Immunocomb® II HIV I and II BiSpot test

Principle of the immunocomb® ii hiv 1 and 2 bispot test

The ImmunoComb® II HIV 1 and 2 BiSpot kit is a rapid test intended for the qualitative and differential detection of antibodies to human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) in human serum or plasma. It is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ('teeth'). Each tooth is sensitized at three spots:

- Upper spot: Goat antibodies to human immunoglobulin (internal control).

- Middle spot: HIV-2 synthetic peptides.
- Lower spot: HIV-1 synthetic peptides.

The developing plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test was performed stepwise, by moving the card from row to row, with incubation at each step.

Test procedure

Antigen–antibody reaction (row a of the developing plate)

- 50 µl of specimen was pipetted using a precision pipette.
- The foil cover of one well in row A of the developing plate was perforated with the perforator and the specimen was dispensed at the bottom of the well.
- It was mixed by repeatedly refilling and ejecting the solution with the pipette, and the pipette tip was discarded.
- The above procedure was repeated for other specimens, including one positive and one negative control supplied with the kit. A new well was used in row A and the pipette tips were changed for each specimen or control.
- The card was inserted into the wells of row A containing specimens and controls, then partially withdrawn and inserted in the wells several times to mix.
- The card was left in row A for exactly 10 minutes and mixed an additional two times during the incubation. Near the end of 10 minutes, the foil of row B was perforated using the perforator.
- At the end of 10 minutes, the card was taken out of row A. Adhering liquid was absorbed from the pointed tips of the teeth on clean absorbent paper.

First wash (Row B)

- The card was inserted into the wells of row B and agitated by vigorously withdrawing and inserting the card in the wells for at least 10 seconds to achieve proper washing.
- The agitation was repeated several times during the course of 2 minutes; meanwhile the foil of row C was perforated. After 2 minutes, the card was withdrawn and the adhering liquid was absorbed.

Binding of conjugate (Row C)

- The card was inserted into the wells of row C, was mixed several times, and allowed for 10 minutes and mixed an additional 2 times during the incubation.
- The foil of row D was perforated. After 10 minutes, the card was withdrawn and the adhering liquid absorbed

Incubation in Conjugate (Row D)

- The card was inserted into the wells of row D and repeatedly agitated during 2 minutes, meanwhile the foil of row E was perforated. After 2 minutes, the card was withdrawn and the adhering liquid absorbed.

Second Wash (Row E)

- The card was inserted into the wells of row E and repeatedly agitated during 2 minutes; meanwhile the foil of row F was perforated. After 2 minutes, the card was withdrawn and the adhering liquid absorbed.

Colour Reaction (Row F)

- The card was inserted into the wells of row F, then withdrawn and inserted several times to mix. After 10 minutes, the card was withdrawn.

Stop Reaction (Row E)

- The card was inserted again into row E. After 1 minute, the card was withdrawn and allowed to dry in the air.

Developing plate

- The used wells were sealed with wide adhesive tape so that nothing could spill out of the wells, even if the Developing Plate was tipped over.

Other kit materials

- The remaining developing plate(s), card(s), perforator, controls, and instructions were returned to the original kit box and stored in an insulated box with ice cubes.

Interpretation of test results

- Upon completion of the test, the tooth used with the positive control usually showed 3 gray-blue spots, while that used with the negative control showed only the upper spot, which should be present on all the other teeth, to confirm that the kit was functioning properly and the test was performed correctly.
- The sole appearance of the upper spot (Internal Control) indicated that the specimen was non-reactive for antibodies to HIV-1 or HIV-2.
- A circular, coloured middle spot indicated the presence of antibodies to HIV-2.
- A circular, coloured lower spot indicated the presence of antibodies to HIV-1.
- Sometimes, high concentrations of either anti-HIV-1 or anti-HIV-2 antibodies produced a faint secondary spot, in addition to the more intense major spot obtained with the homologous antigen (see Figure 8d for high HIV-1 concentration).
- In cases of HIV-1/HIV-2 co-infection, two spots of equal intensities were observed.

ELISA testing (murex HIV ag/ab combination assay)

All the samples were confirmed with enzyme-linked immunosorbent assay, the Murex HIV Ag/Ab combination assay. Murex HIV Ag/Ab combination assay is an enzyme immunoassay for improved detection of seroconversion to HIV-1, HIV-1 group O and detection of anti-HIV-2 antibodies.

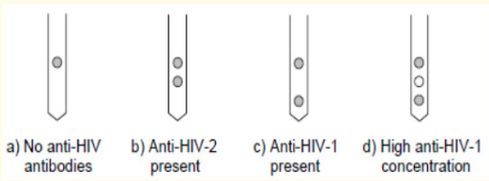

Figure 3: Interpretation of Immunocomb® II HIV I and II BiSpot test results.

Principle of ELISA (MUREX)

Murex HIV Ag/Ab combination is based on microwells coated with synthetic peptide representing immunodominant regions of HIV-1(O) and HIV-2, recombinant proteins derived from the envelope regions of HIV-1 and HIV-2 and HIV pol protein, together with monoclonal antibodies raised against p24 of HIV-1.The conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labelled with horseradish peroxidase.

Tests specimens and control sera are incubated in the wells and reactive HIV core and/or antibodies to HIV in the sample or control sera bind to the antibodies and/or antigens on the microcell; sample and any excess antibodies are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV core and /or specific antibody already bound to the reagents on the well. Samples not containing reactive core antigen or specific antibody will not cause the Conjugate to bind to the well.

Unbound Conjugate is washed away and a solution containing 3, 3', 5, 5 tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue green colour, which is converted to an orange colour, which may be read at 450nm after the reaction has been stopped with sulphuric acid.

In order to prepare the HIV confirmatory screening using Murex HIV Ag/Ab combination assay, the following materials and routine procedure was used:

Materials

- Coated Wells (A plate of 96 microwells coated with HIV antigens and monoclonalantibodies)
- A bottle containing 18ml of a green/brown buffer solution, bovine and murine protein, detergent and saponin labeled Sample Diluent.
- A bottle containing 1.1ml of HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried labelled Conjugate.
- A bottle containing 22ml of a yellow solution consisting of buffer, bovine protein, saponin and detergent labelled Conjugate Diluent.
- Anti-HIV-1, Anti-HIV-2 and HIV-1 p24 Positive Control.
- Negative Control (2 bottles containing 2.5ml of normal human serum).
- Substrate Diluent (A bottle containing 35ml of a colourless solution of tri-sodium citrate and hydrogen peroxide).
- Substrate Concentrate (One bottle containing 35ml of 3,3',5,5'-tetramethylbenzidine (TMB) and stabilisers in an orange solution).
- Stop Solution (0.5M to 2M Sulphuric Acid).
- Freshly distilled or high quality deionized water.
- Micropipettes and Multichannel micropipettes of appropriate volume.
- Incubator capable of maintaining the temperature limits defined in the assay protocol.
- Moulded Heating Block.
- Instrumentation (automated micro plate strip-washer and micro plate reader or fully automated micro plate processor).
- Disposable Reagent Troughs.
- Sodium hypochlorite.
- Sodium hydroxide solution.

Test procedure

- The conjugate was reconstituted and mixed, the substrate solution and wash fluid were prepared.
- 25 µl of sample diluent was added to the number of wells required for the test, with caution not to touch the tops or the bottoms of each well.
- 100 µl of samples or 100 µl controls were added to the wells. For each plate the first column of wells were used for the assay controls. The controls were added to the designated wells after dispensing the samples.
- 100 µl of the negative control was pipetted into each of three wells A1 to C1 and 100 µl of the p24, anti-HIV-1 and HIV-2 and Positive Controls into wells D1 to F1 respectively. A white background was used to aid visualisation of sample addition.
- The wells were covered with the lid and incubated for 60 mins at 37°C. At the end of the incubation time, the plates were washed using an automated strip washer.
- Immediately after washing the plate, 100 µl of conjugate was added to each well, covered with the lid and incubated for 30 mins at 37°C.
- At the end of the incubation time, the plate was again washed using the automated strip washer.
- Immediately after washing the plate, 100 µl of substrate solution was added to each well, covered with a lid and incubated for 30 mins at 37°C, away from direct sunlight. A blue green colour developed in wells containing reactive samples.
- 50 µl of stop solution (0.5M to 2M sulphuric acid) was added to each well.
- Within 15 minutes the absorbance was read at 450 nm using 620nm to 690nm as the reference wavelength.

Calculation of results

The mean absorbance of the negative controls was calculated. If one of the negative control wells had an absorbance more than 0.15 optical density (O.D) above the mean of all three, that value was discarded and the new negative control mean was calculated from two remaining replicates. The cut-off value was calculated by adding 0.150 to the mean of the negative control replicates.

Quality control

Results of the assay were valid if the following criteria for the Controls were met:

- The negative controls had a mean absorbance less than 0.15.
- The absorbance of each of the positive controls was more than 0.8 above the mean absorbance of the negative control.
- Assays which did not meet these criteria were repeated.

Interpretation of results

- **Non-reactive Results:** Samples giving an absorbance less than the cut-off value were considered negative in the assay.
- **Reactive Results:** Samples giving an absorbance equal to or greater than the cut-off value were considered initially reactive in the assay.

HTLV Screening

HTLV testing with ImmunoComb® II HTLV 1 and 2

The ImmunoComb® II HTLV 1 and 2 is a rapid test intended for the qualitative detection of IgG antibodies to HTLV type 1 and HTLV type 2 in human serum or plasma.

Principle of determine test

The ImmunoComb® II HTLV 1 and 2 test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at two spots:

- **Upper spot:** Human immunoglobulin antibodies (Internal Control)
- **Lower spot:** HTLV 1 and 2 recombinant proteins.

The Developing Plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the Card from row to row, with incubation at each step. To start the test, serum or plasma specimens are added to the diluent in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Anti-HTLV antibodies, if present in the specimens, will specifically bind to the recombinant proteins on the lower spots on the teeth of the Card. Simultaneously, immunoglobulins present in the specimens will be captured by the human immunoglobulin antibodies on the upper spot (Internal Control). Unbound components are washed away in row B. In row C, the specific immunoglobulins captured on the teeth will react with anti-human IgG antibodies labeled with alkaline phosphatase (AP). In the next two rows, unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results are visible as gray-blue spots on the surface of the teeth of the Card.

Test procedure

Equipment Needed

- Precision pipette with disposable tips
- Scissors
- Stop watch.

Preparing the Test

All components, developing plates, cards, reagents and specimens were brought to room temperature and the test was performed at room temperature.

Preparing the developing plate

1. The Developing Plate was incubated in an incubator at 37°C for 20 minutes; or left at room temperature for 3 hours.
2. The work table was covered with absorbent tissue to be discarded as biohazardous waste at the end of the test.
3. The reagents were mixed by shaking the Developing Plate.

Preparing the Card

The aluminum pouch of the Card was torn at the notched edge and the card removed without touching the teeth.

Test instructions

Antigen–antibody reaction (row a of the developing plate)

1. 10 µl of serum specimen was pipetted. The foil cover of one well in row A of the Developing Plate was perforated with the perforator and the specimen was dispensed at the bottom of the well and mixed by repeatedly refilling and ejecting the solution with the precision pipette. The pipette tip was discarded.
2. Step 1 was repeated for the other specimens, including one positive and one negative control supplied with the kit. A new well in row A was used and pipette tips changed for each specimen or control.
3. The Card was inserted into the wells of row A containing specimens and controls and mixed by withdrawing and inserting the Card in the wells several times.
    - The Card was left in row A for exactly 10 minutes and mixed an additional two times during the incubation. Near the end of 10 minutes, the foil of row B was perforated using the Perforator to open more wells needed.
    - At the end of 10 minutes, the Card was taken out of row A.
    - Adhering liquid from the pointed tips of the teeth was absorbed on clean absorbent paper without touching the front surface of the teeth.

First Wash (Row B)

The Card was inserted into the wells of row B and agitated vigorously by withdrawing and inserting the Card in the wells for at least 10 seconds to achieve proper washing. Agitation was repeated several times during the course of 2 minutes; meanwhile the foil of row C was perforated. After 2 minutes, the Card was withdrawn and adhering liquid was absorbed.

Binding of Conjugate (Row C)

The Card was inserted into the wells of row C and mixed several times. The card was left for 10 minutes in row C while mixing. The foil of row D was perforated near the end of the 10 minutes. After 10 minutes, the Card was withdrawn and adhering liquid absorbed.

Second Wash (Row D)

The Card was inserted into the wells of row D and repeatedly agitated during 2 minutes, as in step 4. Meanwhile, the foil of row E was perforated. After 2 minutes, the Card was withdrawn and adhering liquid absorbed.

Third Wash (Row E)

The Card was inserted into the wells of row E and repeatedly agitated during 2 minutes. Meanwhile, the foil of row F was perforated. After 2 minutes, the Card was withdrawn and adhering liquid absorbed.

Color Reaction (Row F)

The Card was inserted into the wells of row F and mixed. The card was left for 10 minutes while mixing. After 10 minutes, the Card was withdrawn.

Stop Reaction (Row E)

The Card was inserted again into row E. After 1 minute, the Card was withdrawn and allowed it to dry in the air.

Waste disposal

Used Developing Plates, pipette tips, absorbent paper, and gloves were disposed as biohazardous waste.

Test Results

Validation

In order to confirm that the test functioned properly and to demonstrate that the results were valid, the following three conditions must be fulfilled:

1. The Positive Control must produce two spots on the Card tooth.
2. The Negative Control must produce an upper spot (Internal Control) and no other spot.
3. Each specimen tested must produce an upper spot (Internal Control).

If any of the three conditions were not fulfilled, the results were invalid, and the specimens and controls were retested.

Interpretation of the results

- The sole appearance of the upper spot (Internal Control) indicated that the specimen was non-reactive for antibodies to HTLV 1 and 2.
- A circular lower spot indicated the presence of antibodies to HTLV 1 and 2. Thus, it does not discriminate between HTLV-1 and 2.

Data entry and validation

Baseline data at recruitment was recorded into enrolment forms while complete data including results of laboratory analysis was documented in case report forms. Visual checking for obvious errors and inconsistencies in data was done. All data was entered into a Microsoft Excel 2013 spread sheet and validated.

Data analysis

Data was exported from Microsoft Excel 2013 to the Statistical Package for Social Sciences (SPSS) version 20.0 for analysis. Proportions were compared using the Chi Square ($\chi 2$) test. Results were presented as frequencies or mean, percentages, standard deviation, risk ratio, confidence intervals and p-values. All p values less than 0.05 were considered statistically significant.

Results and Discussions

Results and discussion must illustrate and interpret the reliable results of the study.

Results

Socio-demographic characteristics of the population

Overall, there were 157 participants included in this study from the population in the Abang Minko'o area of the South Region of Cameroon.

Age distribution of participants

The ages of the participants ranged from 15 to 77 years with a mean age of 32.20 ± 12.63 years. The age group of young adults of 20-29 years was most represented (32%) while that of ≥60 years was least represented (3%) (Figure 4).

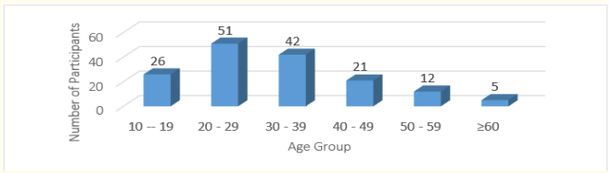

Figure 4: Age Distribution of participants.

Sex distribution of participants

Out of the 157 participants, 70 (45%) were females and 87 (55%) were males giving a sex ratio of female to male of 0.804 (Figure 5).

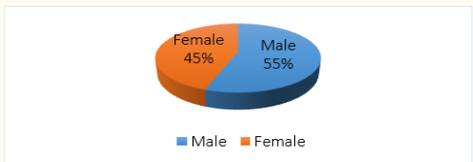

Figure 5: Distribution of participants by sex.

Marital status of participants

As shown on figure 6 below, the majority of our participants were married (41%) while up to 17% admitted to be co-habiting. A large proportion of the population was single (37%) meanwhile 4% was widowed and 1% divorced.

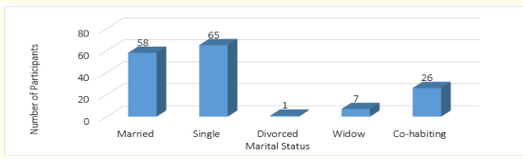

Figure 6: Distribution of participants by marital status.

Occupational distribution

Our participants were involved in various occupations ranging from skilled, semi- skilled to unskilled labour. The majority of the study population were traders (22.9%), followed by students (19.7%), farmers (16.6%), civil servants (12.1%), housewives (4.5%), hairdressers (3.2%) and technicians (1.9%). However, 2.5% were unemployed and 16.6% were classified as "Other" occupations which included: drivers, welders, brick layers, bakers and tailors (Figure 7).

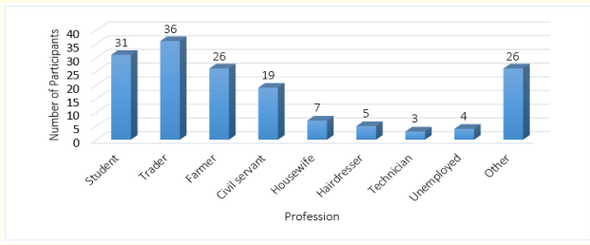

Figure 7: Distribution of participants by occupation.

Religious groups

A majority of the participants were Protestants (64%), followed by Catholics (26%) while Muslims and Pagans were least represented with 5% each (Figure 8).

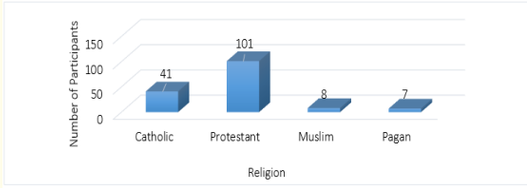

Figure 8: Distribution of participants by religion.

Ethnic groups

More than half of the participants were natives from Abang Minko'o called the Ntoumous (52.9%), while there were also participants from other ethnic groups like Bulu, Bamileke, Beti, and Nso. Other participants from different ethnic groups in Cameroon and other countries like Gabon, Equatrorial Guinea and Congo were classified as 'Other' (Figure 9).

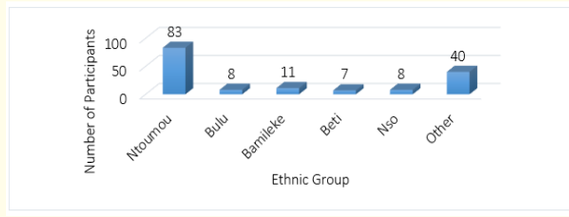

Figure 9: Distribution of participants by ethnic group.

Level of education

All of our participant had a certain level of education. Most of our participants had a high school qualification (45%) as highest level of education while the least represented was a primary education (5%) (Figure 10).

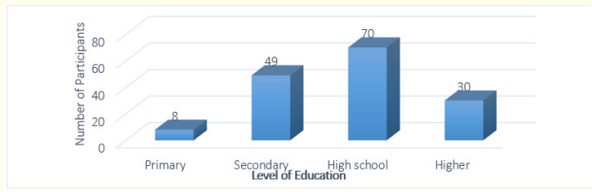

Figure 10: Distribution of participants by level of education.

Results of serologic analysis

Of the 157 samples tested, 5 were positive with Determine HIV 1/2 rapid test. The 5 samples were further tested with Immuno Comb HIV 1 and 2 bi Spot rapid test and they all reacted to this test. All the samples were further tested with Murex HIV Ag/Ab as confirmatory test and 10 samples were found to be reactive to HIV-1 antibodies (Table 1).

Results	Test			Conclusion (n)
	Determine HIV ½	ImmunoComb HIV 1 and 2 biSpot	Murex HIV Ag/ Ab	
Reactive	5	5	10	10
Non-reactive	152	-	147	147
Total	157	5	157	157

Table 1: Prevalence of HIV with various test strategies.

Prevalence of HIV

This led to the final conclusion that 10 samples were reactive out of the 157 samples and the prevalence of HIV was then calculated at 6.4% (Table 2). However, when considering only the age group 15-49 years, the prevalence of HIV was calculated at 5.0% (Table 3).

	HIV status		Total
	Positive	Negative	
N	10	147	157
%	6.4	93.6	100.0

Table 2: Prevalence of HIV in Abang Minko'o.

	HIV status		Total
	Positive	Negative	
N	7	132	139
%	5.0	95.0	100.0

Table 3: Prevalence of HIV in age group 15-49 years in Abang Minko'o.

Prevalence of HIV infection with socio-demographic characteristics

Prevalence of HIV infection with age groups

The HIV infection was most prevalent amongst the age group of 50-59 years (25.0%) followed by 30-39 years (9.5%) while the least prevalent groups were 20-29 years (2.0%) and ≥ 60 years (0.0%). The prevalence of HIV generally increased with age from 20-59 years; however, we noticed a significant decrease after the age of 60 years although it was not statistically significant (p = 0.301) (Figure 11).

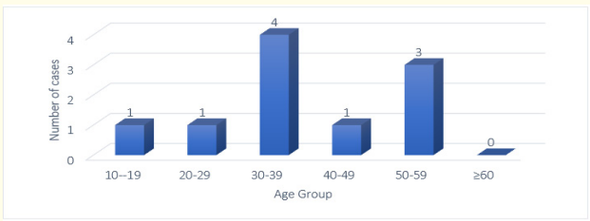

Figure 11: Prevalence of HIV with age groups.

Prevalence of HIV infection with sex

The prevalence of HIV infection in females (7.1%) was slightly greater than that in males (5.7%). However, the difference was not statistically significant (p = 0.722) (Table 4).

Sex	HIV+ cases	p-value	Risk Ratio	95 % Confidence interval
		0.722		
Male	5 (5.7%)		1	
Female	5 (7.1%)		1.131	0.592 - 2.157

Table 4: Prevalence of HIV by sex.

Prevalence of HIV inefction with marital status

Those who were married had the highest prevalence of HIV (8.6%), followed by those who were co-habiting (7.7%). The least prevalence was noted amongst those who were widowed and those who were divorced (0.0%). However, the association between HIV infection and marital status was not statistically significant (p = 0.836) as shown in table 5.

Marital status	HIV+ cases	P-value
		0.836
Married	5 (8.6%)	
Single	3 (4.6%)	
Co-habiting	2 (7.7%)	
Divorced	0 (0.0%)	
Widowed	0 (0.0%)	

Table 5: Prevalence of HIV by marital status.

Prevalence of HIV with occupation

Housewives had the highest prevalence of HIV by profession (28.6%), followed by the unemployed (25.0%) meanwhile the lowest prevalence was recorded amongst hairdressers, technicians and those in other occupations (0.0%). However the association between HIV infection and occupation was not statistically significant (p = 0.170) (Table 6).

Occupation	HIV+ cases	P-value
		0.170
Student	1 (3.2%)	
Trader	2 (5.6%)	
Farmer	2 (7.7%)	
Civil servant	2 (10.5%)	
Housewife	2 (28.6%)	
Hairdresser	0 (0.0%)	
Technician	0 (0.0%)	
Unemployed	1 (25.0%)	
Other	0 (0.0%)	

Table 6: Prevalence of HIV by occupation.

Prevalence of HIV infection with level of education

The participants with secondary education had the highest prevalence of HIV infection by highest level of education (8.2%) and the least represented were those with primary education (0%). However, the association between HIV infection and the highest level of education was not statistically significant (p-value = 0.834) (Table 7).

Level of Education	HIV+ cases	p-value	Risk Ratio	95% Confidence interval
		0.834		
Primary	0 (0.0%)		1	
Secondary	4 (8.2%)		1.178	1.051-1.319
High school	4 (5.7%)		1.121	1.036-1.214
Higher	2 (6.7%)		1.286	1.080-1.531

Table 7: Prevalence of HIV by level of education.

Prevalence of HIV infection with religion

The prevalence of HIV infection was highest amongst Catholics (7.3%) and least among Muslims and pagans (0%). However, the association between HIV infection and religion was not statistically significant (p-value = 0.834) (Table 8).

Religion	HIV+ cases	p-value	Risk Ratio	95% Confidence interval
Catholic	3 (7.3%)	0.769	1.184	1.045-1.342
Protestant	7 (6.9%)		1.074	1.019-1.133
Muslim	0 (0.0%)		-	-
Pagan	0 (0.0%)		1	

Table 8: Prevalence of HIV by religion.

Prevalence of HIV infection with ethnic group

Participants from Ntoumou had the highest prevalence of HIV infection by ethnic group (8.4%) while the least represented were those from Bamileke, Bulu, Beti and Nso (0.0%). However, the association between HIV infection and ethnic group was not statistically significant (p-value = 0.701) (Table 9).

Ethnic group	HIV+ cases	P-value
		0.701
Ntoumou	7 (8.4%)	
Bulu	0 (0.0%)	
Bamileke	0 (0.0%)	
Beti	0 (0.0%)	
Nso	0 (0.0%)	
Other	3 (7.5%)	

Table 9: Prevalence of HIV by ethnic group.

Prevalence of HTLV

Prevalence of HTLV in abang minko'o

Out of the 157 participants who met our inclusion criteria, 35 were seropositive for HTLV giving a prevalence rate of 22.3% (Table 10).

	HTLV status		Total
	Negative	Positive	
N	122	35	157
%	77.7	22.3	100

Table 10: Prevalence of HTLV in Abang Minko'o.

Prevalence of HTLV with socio-demographic characteristics

Prevalence of HTLV with age groups

The prevalence of HTLV was highest amongst the age group 50-59 years (41.7%) followed by the age group 20-29 years (27.5%) and lowest amongst the age group 10-19 years (15.4%). A trough in HTLV prevalence was noted between the age group 30-49 years and a significant decrease in individuals of ≥60 years after the highest peak. However, the association between HTLV infection and age group was not statistically significant (p = 0.550) (Figure 12).

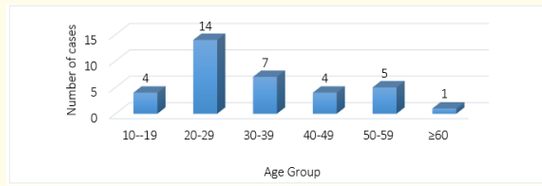

Figure 12: Prevalence of HTLV by age groups.

Prevalence of HTLV with sex

The prevalence of HTLV infection in males (26.4%) was greater than that in females (17.1%), though the difference was not statistically significant (p = 0.164) (Table 11).

	HTLV+ cases	Total	p-value	Risk Ratio	95%CI
			0.164		
Male	23 (26.4%)	87		1.253	0.935-1.679
Female	12 (17.1%)	70		1	
Total	35	157			

Table 11: Prevalence of HTLV by sex.

Prevalence of HTLV with marital status

Widows had the highest HTLV prevalence rate by marital status (28.6%) followed by those who were co-habiting (26.9%) while the divorced recorded the lowest prevalence rate (0.0%). However, the association between HTLV prevalence and marital status was not statistically significant (p = 0.813) (Table 12).

Marital Status	HTLV+ Cases	Total	P-Value
			0.813
Married	15 (25.9%)	58	
Single	11 (16.9%)	65	
Divorced	0 (0.0%)	1	
Widowed	2 (28.6%)	7	
Co-habiting	7 (26.9%)	26	
Total	35	157	

Table 12: Prevalence of HTLV by marital status.

Prevalence of HTLV with occupation

Farmers had the highest HTLV prevalence by occupation (38.5%) followed by individuals in other occupations (34.6%) while hairdressers recorded the lowest prevalence rate (0.0%), but the association between HTLV status and occupation was not statistically significant (p = 0.127) (Table 13).

Prevalence of HTLV with highest level of education

The prevalence of HTLV infection was most amongst participants with primary education as highest level of education (25.0%) and least amongst those with higher education (16.7%). However, the association between HTLV infection and highest level of education was not statistically significant (p = 0.453) (Table 14).

Occupation	HTLV+ Cases	Total	P-Value
			0.127
Student	5 (16.1%)	31	
Trader	5 (13.9%)	36	
Farmer	10 (38.5%)	26	
Civil servant	3 (15.8%)	19	
Housewife	1 (14.3%)	7	
Hairdresser	0 (0.0%)	5	
Technician	1 (33.3%)	3	
Unemployed	1 (25%)	4	
Other	9 (34.6%)	26	
Total	35	157	

Table 13: Prevalence of HTLV by occupation.

Level of Education	HTLV+ Cases	Total	P-Value
			0.453
Primary	2 (25.0%)	8	
Secondary	12 (24.5%)	49	
High School	16 (22.9%)	70	
Higher	5 (16.7%)	30	
Total	35	157	

Table 14: Prevalence of HTLV by highest level of education.

Prevalence of HTLV with religion

The prevalence of HTLV was highest amongst Catholics (29.3%) and least amongst Muslims (12.5%). However the association between HTLV and religion was not statistically significant (p = 0.485) (Table 15).

Religion	HTLV+ Cases	Total	P-Value
			0.485
Catholic	12 (29.3%)	41	
Protestant	20 (19.8%)	101	
Muslim	1 (12.5%)	8	
Pagan	2 (28.6%)	7	
Total	35	157	

Table 15: Prevalence of HTLV by religion.

Prevalence of HTLV with ethnic group

The prevalence of HTLV was highest amongst participants from the Nso ethnic group (37.5%) and lowest amongst the Betis (0.0%). However, the association between HTLV infection and ethnic group was not statistically significant (p = 0.800) (Table 16).

Ethnic Group	Htlv+ Cases	Total	P-Value
			0.800
Ntoumou	20 (24.1%)	83	
Bulu	2 (25.0%)	8	
Bamileke	1 (9.1%)	11	
Beti	0 (0.0%)	7	
Nso	3 (37.5%)	8	
Other	9 (22.5%)	40	
Total	35	157	

Table 16: Prevalence of HTLV by ethnic group.

Co-infection of HIV and HTLV

Prevalence of HIV and HTLV co-infection

Out of the 157 participants tested, dual infection with HIV and HTLV 1 and 2 was noted in 4 participants giving a prevalence of co-infection of 2.5% in the study population. The prevalence of HTLV 1 and 2 infection in HIV-1 seropositive participants was 40.0% while that in HIV negative participants was 21.1%, a difference that was statistically insignificant. This showed that the proportion of HTLV infection in HIV positive participants was increased by almost 2 folds when compared to HIV negative participants (40.0% versus 21.1%). As a measure of the strength of association between HTLV and HIV infections, the Risk ratio calculated was 2.324 (95% CI 0.694-7.778). However, there was no significant association between HTLV and HIV infections (p = 0.164) (Table 17).

HIV status	HTLV status		Total
	Negative	Positive	
Negative	116 (78.9%)	31 (21.1%)	147 (100.0%)
Positive	6 (60.0%)	4 (40.0%)	10 (100.0%)
Total	122 (77.7%)	35 (22.3%)	157 (100.0%)

Table 17: Prevalence of HIV and HTLV co-infection in Abang Minko'o.

Socio-demographic characteristics of HIV and HTLV co-infected participants

As shown on table 18 below, HIV-1 and HTLV 1 and 2 co-infection was found only amongst participants from the Ntoumou ethnic group and the majority of them were married (75%). Males were more infected than females (sex ratio 3:1).

Age	Sex	Marital status	Profession	Highest level of Education	Religion	Ethnic group
32	Male	co-habiting	Unemployed	High school	Protestant	Ntoumou
39	Female	Married	Farmer	Secondary	Protestant	Ntoumou
53	Male	Married	Civil servant	Higher	Catholic	Ntoumou
54	Male	Married	Farmer	Secondary	Protestant	Ntoumou

Table 18: Characteristics of HIV-1 and HTLV 1 and 2 co-infected participants.

Distribution of HIV and HTLV infections with age groups

As shown on figure 13 below, the frequency of HTLV 1 and 2 and HIV infections amongst seropositive participants for each virus was highest within the age group of 30-39 years, while the lowest frequency was observed amongst those ≥60 years. We also observed an equal distribution of both infections amongst the age groups 10-19 years and 40-49 years. The figure below also shows a general decrease in the frequency of HTLV 1 and 2 and HIV infections from the 30-39 years to the ≥ 60 years age groups.

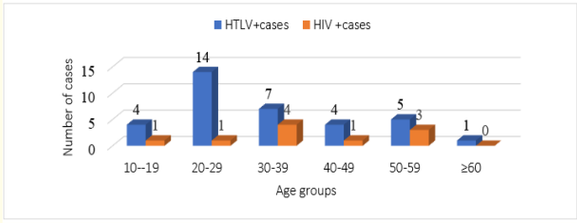

Figure 13: Distribution of HIV and HTLV infections by age groups.

As shown on figure 14 below, the trends of HIV and HTLV 1 and 2 infections amongst seropositive participants for each virus in the age groups of our study population were almost similar, with highest peaks for each virus in young adults between 20 - 39 years.

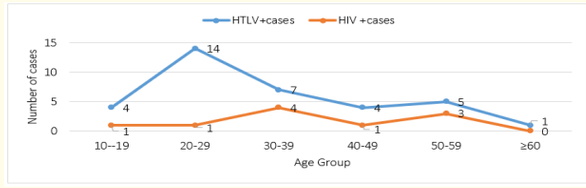

Figure 14: Pattern of HIV and HTLV infections by age groups.

Risk factors associated with sexual behaviour and HIV infection

Distribution of factors associated with risky sexual behaviour amongst participants and HIV status

Behaviours such as sex-money exchange (prostitution) and a history of STIs were significantly associated with HIV infection with p-values of 0.039 and 0.048 respectively. Those who practised sex-money exchange were almost 2 times more exposed to HIV infection than those who did not (RR 1.729; p = 0.039). In the same light, participants who had a history of STIs were 2 times more exposed to HIV infection (RR 2.005; p = 0.048). However, other characteristics identified such as alcohol consumption, cigarette consumption, regular visiting of night clubs or bars, sexual activity, age at first sex, number of lifetime partners and partners in the last 12 months, cheating on partner, use of condoms, same gender sex and a history of induced abortion or unwanted pregnancy in women, had no statistically significant association with HIV infection (Table 19).

Age	Sex	Marital status	Profession	Highest level of Education	Religion	Ethnic group
32	Male	co-habiting	Unemployed	High school	Protestant	Ntoumou
39	Female	Married	Farmer	Secondary	Protestant	Ntoumou
53	Male	Married	Civil servant	Higher	Catholic	Ntoumou
54	Male	Married	Farmer	Secondary	Protestant	Ntoumou

Table 18: Characteristics of HIV-1 and HTLV 1 and 2 co-infected participants.

Frequency distribution of risky sexual behaviours and HIV status

As shown on table XXI below, the frequency of behaviours such as visits to night clubs or bars, the use of condoms, sex-money exchange and same gender sex were not significantly associated with HIV infection. However, those who visited night clubs or bars regularly were 2 times more exposed to HIV infection (RR 2.066; p = 0.506) when compared to those who never visited such places. Also, participants who practised sex-money exchange sometimes were almost 2 times more exposed to HIV infection (RR 1.772; p = 0.075) and those who did so regularly were 4 times more exposed to HIV infection (RR 4.450) when compared to those who never engaged in such practice. Same gender sex (homosexuality) also exposed more to HIV infection with almost 4 times more risk to contract HIV infection (RR 3.675; p = 0.205) in participants engaged in this practice when compared to those who did not engage in such practice.

The use of condoms was found to be protective against HIV infection and those who used condoms consistently were even more protected (RR 0.000; p = 0.554) than those who did so inconsistently (RR 0.854; p = 0.554) when compared to those who did not use condoms at all (Table 20).

Justification of sexual behaviour of participants and HIV status

Whatever reason given by the participants for not having sex, cheating on their partners or not using condoms regularly was not significantly associated with HIV infection However, those who cheated on their partners under the influence of alcohol or cigarette or drug consumption were 2 times more exposed to HIV infection (RR 2.067; p = 0.332) when compared to those who did so just because the opportunity presented (Table 21).

Frequency of Factor	HIV+ Cases	% HIV+ Cases/Total Population	Risk Ratio	95% CI
Night club or bar visit (p=0.506)				
Never	5 (5.8%)	3.18	1	
Sometimes	3 (5.4%)	1.91	0.948	0.378-2.376
Regularly	2 (13.3%)	1.27	2.066	0.577-7.396
Condom use (p=0.554)				
Never	5 (8.2%)	3.18	1	
Sometimes	5 (6.0%)	3.18	0.854	0.452-1.614
Always	0 (0.0%)	0.00	0.000	-
Sex-money exchange (p=0.075)				
Never	2 (2.5%)	1.27	1	
Sometimes	6 (9.4%)	3.82	1.772	1.135-2.765
Regularly	2 (16.7%)	1.27	4.450	1.422-13.925
Same gender sex (p=0.205)				
Never	9 (5.9%)	5.73	1	
Sometimes	1 (20.0%)	0.64	3.675	0.452-29.879
Regularly	0 (0.0%)	0.00	-	-

Table 20: Frequency of risky sexual behaviours associated with HIV infection.

Justification Of Behaviour	HIV+ Cases	% HIV+ Cases/Total Population	Risk Ratio	95% Confidence Interval
Reason for not having sex				
Fear of HIV and other STIs	0 (0.0%)	0.00	-	-
Religious reason	0 (0.0%)	0.00	-	-
Waiting marriage	0 (0.0%)	0.00	-	-
Reason for cheating on partner (p=0.332)				
Alcohol or drugs or cigarette consumption	2 (25.0%)	1.27	2.067	0.568-7.521
Distance from partner	2 (5.0%)	1.27	0.829	0.363-1.891
Peer pressure	1 (7.7%)	0.64	0.771	0.133-4.480
Opportunity presented	3 (10.7%)	1.91	1	
Reason for no condom use (p=0.465)				
Lack of information	1 (25.0%)	0.64	1	
Cultural taboo	2 (12.5%)	1.27	0.810	0.353-1.856
Religious reason	1 (4.3%)	0.64	0.568	0.141-2.289
Fear of side effects	1 (16.7%)	0.64	0.800	0.181-3.536
Decrease pleasure or satisfaction	1 (3.3%)	0.64	0.551	0.137-2.216
Not available when needed	4 (6.1%)	2.55	0.839	0.539-1.304

Table 21: Justification of sexual behaviour and HIV prevalence.

Discussions

Characteristics of the study population

Our study involved only adult individuals aged 15 years and above who were regular visitors or residents of the Abang Minko'o area. We had 157 participants; higher than our minimum acceptable sample size calculated at 103. The mean age of our study group was 32 years (range 15-77 years) and the age group of 20-29 years (young adults) was most represented (32%). Males were more represented (55%) than females (45%). This is discordant with the gender distribution in Cameroon, which is made of 51% of females and 49% males [35] probably because males flood this commercial area from other parts of the country to improve the standard of living of their families back home. The most represented age group was similar to the results from the Cameroon Demographic Health Survey (DHS) in 2011 [35] where the age group of 20-29 years was most represented (36.8%) in the general population. The predominance of the 20-29 years age group could be explained by the fact that this group comprised young adults who were quick to respond to beneficial opportunities like free screening tests in the community.

The majority of our participants were married (41%), while up to 17% admitted to be co-habiting. The high level of co-habitation could be explained by the rural setting with low level of education and income which favours such practice. Most of our participants were involved in trading (22.9%) followed by students (19.7%) and farmers (16.6%). This could be explained by the main activity of our study area which serves as a border market for the exchange of commodities between Cameroon and Gabon. Also, the population in this area thrive mainly on agriculture and commerce, and students found in this area are made of permanent residents of the Abang Minko'o area as well as people from other neigbouring villages in Gabon who study in this area while residing in Gabon.

A majority of our participants were Protestants (64%) which could be explained by the presence of numerous protestant churches in this area like the Baptist, Presbyterian and Pentecostal churches, followed by Catholics (26%) and a few Muslims (5%). This is similar to the religious distribution of the Ambam Division which has a majority of Christians from Protestant churches and a few Muslims. More than half of our participants were natives from Abang Minko'o called the Ntoumous (52.9%) while the rest were natives from different ethnic groups in Cameroon and other countries like Gabon, Congo and Equatorial Guinea. This could be explained by the commercial character of our study area which attracts people from other areas who reside or regularly visit this area to buy and sell goods. All of our participants had at least primary education with a majority having a high school qualification as highest level of education (45%). This is similar to the high literacy rate in Cameroon estimated at about 71.29% in 2010 [32] The majority of high school qualification could be explained by the presence of numerous high schools in this area but a lack of higher training institutions which could encourage the population to continue their studies.

Prevalence of HIV

Of the 157 participants tested, 10 were HIV infected giving a prevalence rate of 6.4% in our study population in the Abang Minko'o area of the South Region of Cameroon. All the positive cases recorded were HIV type 1 on the ImmunoComb II HIV 1 and 2 BiSpot test. The prevalence was highest in the age group of 50-59 years (25%) followed by the age group of 30-39 years (9.5%). However, when considering only the age group of 15-49 years in our study, this being the adult age group recommended by the UNAIDS Reference Group on Estimates, Modelling and Projections for the purpose of comparing HIV prevalence worldwide (60), 5.0% (7 participants out of 139) were HIV infected. Therefore the prevalence of HIV for the adult age group of 15-49 years in our study population in the Abang Minko'o area was 5.0%. This was slightly less than the prevalence rate reported in the Cameroon DHS in 2011 at 7.2% for the South Region [35]. In our study, the prevalence of HIV infection was higher in females (7.1%), than in males (5.7%). This was similar to the Cameroon DHS results in 2011 where the prevalence of HIV infection was higher in Cameroonian females (10.6%) than in males (3.8%). These results which are comparable, signify that females are more vulnerable to HIV infection than males. The age group of 30–39 years had the highest prevalence of HIV infection (9.5%) with respect to the 15-49 years grouping of our participants, similar to the results of the Cameroon DHS in 2011 where the most prevalent age group was 35-39 years with 8.1% HIV prevalence rate [35].

Those who were married were the most infected by HIV (8.6%) followed by those who were co-habiting (7.7%) then singles (4.6%). These results were discordant to the reports of the Cameroon DHS in 2011 which reported an HIV prevalence of 5% amongst couples and the highest HIV prevalence amongst widows and widowers (16.9%) [35]. This difference could be explained by the behaviour of our participants who admitted to having extra-marital affairs or cheating on their regular partners when the opportunity presented (1.91% of the total population) and also because of distance between them (1.27% of the total population), as they often leave their partners to spend some days in this border area which offers opportunities for risky sexual behaviour. Housewives had the highest HIV prevalence by profession (28.6%) followed by the unemployed (25%) which could be explained by the absence of formal jobs and unemployment amongst this group of people which predispose them to risky activities such as prostitution and other risky behaviours which could increase HIV transmission.

Participants with secondary education were the most infected by HIV (8.2%) which was similar to the Cameroon DHS results in 2011 where participants with secondary education reported the highest HIV prevalence rate (1.9%) [35]. HIV infection was most prevalent amongst Catholics (7.3%) compared to Pagans (0%). These results were discordant to results from the Cameroon DHS in 2011 where Catholics were fourth in terms of HIV prevalence (4.4%) and Pagans last but one with 2.6% [35]. This could be explained by the fact that sexual behaviours are less tempered by religious norms especially in border areas which offer opportunities for risky sexual behaviour [36]. Natives from the Abang Minko'o area, the Ntoumous were the most infected by HIV (8.4%). This could be explained by the fact that this population which is located in a border area is more vulnerable to HIV infection as it receives mobile populations from different ethnic groups and their convergence and mixture offers opportunities for risky sexual behaviours [36].

Prevalence of HTLV

Out of 157 participants who met our inclusion criteria, 35 were seropositive for HTLV giving a prevalence rate of 22.3%. This is in accordance to findings in a study done by Proietti., et al. in 2005 on worldwide prevalence of HTLV which estimated the prevalence of HTLV in adults to be between 2-30% in endemic zones [37]. Mbanya., et al. in 2002 found a lower rate of HTLV-1 (1.6%) infection amongst first time blood donors in Yaounde, Cameroon [21] but this study looked only at HTLV-1 infection whereas our

study took into account both HTLV type 1 and 2. Another study by Ndumbe., et al. in 1993 amongst Pygmies in Cameroon found an HTLV prevalence rate of 10.9% [20] which was less than the prevalence found in our study probably because of the differences in the study populations.

The prevalence of HTLV 1 and 2 was highest amongst the age group 50 - 59 years (41.7%) followed by the young adults of age group 20 - 29 years (27.5%) and lowest amongst the age group 10 - 19 years (15.4%). These findings were similar to results from a study done by Proietti., et al. in 2005 which showed that the prevalence of HTLV increases with age [37].

The prevalence of HTLV infection in males (26.4%) was greater than that in females (17.1%). This was discordant to findings of the study by Proietti., et al. which showed that females were more infected than males [37]. This difference could be explained by the fact that our participants were mostly males and our sample size was relatively smaller.

Prevalence of HIV and HTLV co-infection

Of the 157 participants tested, dual infection with HIV and HTLV 1 and 2 was noted in 4 participants giving a prevalence of co-infection of 2.5% in the study population. The prevalence of HTLV 1 and 2 infection in HIV-1 seropositive participants was 40.0%. This finding was not similar to the prevalence rate reported in a study by Regis., et al. in 2005 which found a prevalence of 5 - 15% of HTLV-1 infection in HIV-1 infected people in the USA, Europe and in some developing countries [38]. However, this same study reported that these rates could be 100-500 times higher than that in the general population. Our study found a 16 times increase between co-infection rates in HIV-1 and HTLV 1 and 2 infected participants and the co-infection rate in the general population, which was discordant to reports by Regis., et al. This difference could be explained by the difference in sample size and because our study looked at both HTLV 1 and 2 infection in HIV-1 infected persons.

The co-infection of HIV-1 and HTLV 1 and 2 was found only amongst participants from the Ntoumou ethnic group and the majority of them were married (75%). Males were more infected than females (sex ratio 3:1). This is not surprising since natives from Abang Minko'o, the Ntoumous reside in the South Region of Cameroon which is an endemic zone for HTLV infection [22] and has also been noted for its high HIV prevalence in the country [35].

The trends of HIV and HTLV 1 and 2 infections in seropositive participants for each virus in the age groups of our study population were almost similar. This could be explained by the fact that these two retroviruses share similar routes of transmission [17].

Risk factors associated with sexual behaviour and HIV transmission

Amongst the risk factors identified, behaviours such as sex-money exchange (prostitution) and a history of STIs were significantly associated with HIV infection with p-values of 0.039 and 0.048 respectively. Those who practised sex-money exchange were almost 2 times more exposed to HIV infection than those who did not (RR 1.729; p = 0.039). Also, those who were regularly involved in sex-money exchange were 4 times more exposed (RR 4.450; p = 0.075) while those who did so sometimes were almost twice at risk of HIV infection (RR 1.772; p = 0.075) when compared to those who did not engage in such practice though this difference was statistically insignificant. This confirms the fact that prostitution constitutes a major risk factor for the transmission of HIV infection and other STIs and that Migrants' multi-local social networks create opportunities for sexual networking [39].

A history of STIs was significantly associated with HIV infection (p = 0.048) in 12% of the HIV infected and participants who had a history of STIs were 2 times more exposed to HIV infection (RR 2.005) compared to those who did not have any history of STIs. This is in accordance with the fact that STIs expose more to HIV infection since they disrupt the natural protective mechanisms of the genital organs. The prevalence of HIV infected participants in the total population who had a history of STIs was 3.82% which was less than that in the Cameroon DHS in 2011 at 7.1% [35]. This could be due to differences in age range and sample size.

Characteristics such as alcohol consumption, cigarette consumption, regular visiting of night clubs or bars, sexual activity, age at first sex, number of lifetime partners and partners in the last 12 months, cheating on partner, use of condoms, same gender sex and a history of induced abortion or unwanted pregnancy in women, had no statistically significant association with HIV infection. However, there were differences between participants who were exposed to these factors when compared to those who were not, even though these differences where statistically insignificant. Those who consumed alcohol were slightly more at risk (RR 1.097; p = 0.786) meanwhile those who smoked cigarettes were almost twice at risk (RR 1.838; p = 0.381) of being HIV infected. This finding is similar to results of a study which found that risk perception ability decreases with alcohol and cigarette consumption, indicating that individuals who use alcohol and other stimulants were more likely to get infected with HIV and other STIs [40]. Also, those who visited bars or night clubs were slightly more at risk of HIV infection (RR 1.114; p = 0.754) and regular visitors were 2 times more exposed to HIV infection (RR 2.066; p = 0.506) compared to those who never visited these places. This indicated that night clubs and bars were one of the sites where HIV infection is likely to be acquired; as a result regular night club or bar attendants were more vulnerable to HIV infection especially since as the frequency of attending night clubs or bars increases, the trend of condom use is likely to decrease.

Characteristics like sexual activity, age at first sex, number of lifetime partners and partners in the last 12 months, and cheating on partner, were not significantly associated with HIV infection in

our study population. Nevertheless, sexually active participants were slightly more at risk to be HIV infected (RR 1.035; p = 0.553) with a slight increase of risk of HIV infection in those who first had sex at less than 16 years (RR 1.216; p = 0.260) when compared to those who first had sex at ≥20 years even though these differences were statistically insignificant. This could be explained by the fact that early sex is likely linked to the tendency of multiplicity of sexual partners, and multiple sexual partners constitutes a major risk factor for HIV transmission. The prevalence of HIV infection in participants who started sexual activity at less than 16 years in the general population was 3.82% which was slightly less than results from the Cameroon DHS in 2011 at 5.5% [35]. The difference could be explained by differences in study populations.

Those who had more than 1 sexual partner in the last 12 months were slightly more at risk of HIV infection (RR 1.421; p = 0.206) compared to those with one partner. This could be explained by the fact that as the number of sexual partners increase, the chances of contracting STIs also increase since the consistent use of condoms is likely to decrease. The prevalence of HIV infected participants in the total population who had more than one sexual partner in the last 12 months was 4.46% which was similar to results from the Cameroon DHS in 2011 at 4.7% [35]. Participants who admitted to have cheated on their partners presented a slight increase in risk of HIV infection (RR 1.452; p = 0.124) compared to those who did not cheat. This could be explained by the fact that cheating implies multiplicity of sexual partners which increases the chances of HIV infection and this was similar to a study which established that mobility per se can encourage or make people vulnerable to high risk sexual behaviours like cheating [39].

Also, women who had performed an induced abortion or had an unwanted pregnancy were more exposed to HIV infection (RR 1.405; p = 0.313) compared to those who had no history of induced abortion or unwanted pregnancy. This is could be explained since unwanted pregnancies which sometimes require induced abortions usually result from risky sexual behaviours or inconsistent use of condoms which expose to HIV infection.

The frequency of behaviours such as the use of condoms and same gender sex were not significantly associated with HIV infection. However, the use of condoms was found to be a protective factor against HIV infection (RR 0.808; p = 0.455) and those who always used condoms were even more protected (RR 0.000; p = 0.554) than those who used it sometimes (RR 0.854; p = 0.554) when compared to those who did not use condoms at all. This confirmed the fact that regular and consistent use of condoms can protect against STIs including HIV infection.

Same gender sex (homosexuality) also exposed more to HIV infection with almost 4 times more risk to contract HIV infection (RR 3.675; p = 0.205) in participants engaged in this practice when compared to those who did not engage in such practice. This indicated that homosexuality was not rare in this border area and constitutes a major risk factor for HIV transmission in individuals who engage in such practice. Also, the participants engaged in homosexual activity were all males confirming the fact that men who have sex with men are more at risk of HIV infection [41].

Whatever reason given by the participants for not having sex, cheating on their partners or not using condoms regularly was not significantly associated with HIV infection. However, those who cheated on their partners under the influence of alcohol or cigarette or drug consumption were 2 times more exposed to HIV infection (RR 2.067; p = 0.332) when compared to those who did so just because the opportunity presented, even though this difference was statistically insignificant. This reason could be explained by the fact that individuals who drink alcohol or consume drugs and other stimulants are likely to be engaged in unprotected sex and this is in accordance to a study which found that alcohol consumption exposed more to HIV infection since alcohol limits the cognitive capacity of the individuals and leads them to have unsafe sex [42].

Conclusions

At the end of this study, we can draw the following conclusions:

- The prevalence of HIV is high in Abang Minko'o area of the South Region of Cameroon (6.4%).
- The prevalence of HTLV 1 and 2 is high in Abang Minko'o area (22.3%).
- Co-infection of HIV and HTLV 1 and 2 is not rare (prevalence of co-infection in Abang Minko'o is 2.5%).
- The prevalence of HTLV 1 and 2 in HIV-1 infection is high (40% that is 4 cases out of 10).
- Prostitution and a history of STIs are the major risk factors associated with sexual behaviour and HIV transmission in Abang Minko'o.
- Sexual behaviours are less tempered by social norms and values in this border area.
- The Abang Minko'o population constitutes a potentially vulnerable group for HIV and HTLV 1 and 2 transmission.

Recommendations

Based on findings and conclusions from this study, we therefore make the following recommendations:

To the ministry of public health

- To emphasize the need for regular and consistent use of condoms especially in mobile populations as a tool to prevent HIV transmission and co-infections.
- To discourage prostitution and homosexuality across the national territory.
- To evaluate the cost-effectiveness of systematic screening for HTLV 1 and 2 on all blood donors before transfusion of collected samples in order to prevent transmission of the virus by blood transfusion, through complementary studies on HTLV nationwide.

To the FMBS and other research institutions

- To carry out studies on HTLV and its co-infection with HIV on larger populations, and also to better identify specific risk factors associated with their transmission in vulnerable population groups like mobile populations in order to implement appropriate prevention strategies.

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Conflict of Interest

Declare if any financial interest or any conflict of interest exists.

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