SEROSURVEY OF HUMAN T-LYMPHOTROPHIC VIRUS TYPE 1 SECONDARY INFECTION AMONG PEOPLE LIVING WITH HIV/AIDS IN DUTSE METROPOLIS, NORTH-WESTERN NIGERIA

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Abstract
Human T-lymphotrophic virus type 1 (HTLV-1) is a causative agent of tropic spastic paraparesis and adult T-cell leukaemia, Evidence is accumulating that HTLV-1 may be responsible for some degree of subclinical immune suppression that may results in increased rate of HIV. Sixty (60) people living with HIV/AIDS consisting of 20 males and 40 females were recruited in this study. HIV diagnosis was confirmed using Nigerian National Serial Algorithm for HIV test. ELISA technique was used for the detection of HTLV-1 IgG and IgM antibodies, and Cyflow partec was used for CD4 count. The prevalence of HTLV-1 IgG and IgM antibodies among HIV subjects was 15% and 6.6% respectively. Male patients have a percentage prevalence of 4(6.6%) and female 5(8.3%) of IgG antibodies, IgM antibodies prevalence was 2(3.3%) for male and female each respectively. CD4 Counts of the HIV subjects was evaluated which reveals that patients with counts 0-200cells/µl tested negative to HTLV-1 IgG and IgM antibodies. **Conclusion:** The percentage prevalence recorded in this study shows that HTLV-1 infection is relatively high compared to the previous studies even though limited information was obtained in relation to HIV/HTLV-1 co-infection in these study area.
Keywords: Human T-lymphotrophic virus -1, HIV/AIDS, CD4,

INTRODUCTION
Human T-Lymphotrophic virus(HTLV-1) is the first human retrovirus to be discovered. The virus has been found to be associated with several disorders such as adult T-cell Luekemia(ATL), trophic spastic paraparesis, HTLV-1 uveitis, and HTLV-1 associated infective dermatitis Gessain and Cassar (2011); Goncalves *et al*. (2010). The ATL develops only in HTLV-1 infected individuals and all ATL cells that contain integrated HTLV-1 provirus (Arisawa *et al., 2000*). HTLV-1 is endemic in many parts of the world, including south-western Japan, some of the Caribbean islands, South America, and foci in western and central Africa and Australo-Melanesia. Epidemiological and molecular studies demonstrate a credible association between HTLV-1 infection and ATLL, which is a rare and aggressive T-cell cancer found most commonly in areas where HTLV-1 is endemic, the risk of developing ATLL was greater with higher proviral load (the percentage of circulating CD4 T cells with integrated viral DNA) or higher anti-HTLV-1 antibody levels. Prospective studies and detection of a monoclonal insertion site of HTLV-1 DNA in tumours indicate that infection precedes diagnosis of ATLL (Biswas *et al.,* 2010; IARC,

2012; Stienlauf *et al.,* 2013). Most HTLV-1-infected individuals are lifelong symptomless carriers [7] only 2% to 5% of infected people develop diseases related to the virus [8]. The present research focuses on determining the prevalence HTLV-1 among HIV patients, as well as finding out the involvement of CD4 cell count with regards to HIV/HTLV-1 coinfection.

MATERIALS METHODS
Study Design and Study Population
This is a descriptive prospective cross- sectional study involving 60 subjects of people living with HIV/AIDS that presented at the ART Clinic of Rasheed Shekoni Specialist Hospital, Dutse, Jigawa state between January to December, 2017.
Laboratory Analysis
Specimen Collection: About 5mls of whole blood was collected aseptically from each subject using standard venepuncture. Samples were dispensed into appropriately labelled screw – capped containers and was left at room temperature for about an hour, after which it was spunat 3,000 rpm for 10minites to separate serum from blood clot. Serum were dispensed into corresponding labelled plain containers and was stored at – 20⁰C until needed for assay

Determination of HIV: HIV status of the subjects for this study were tested and confirmed in accordance with National serial algorithm for HIV testing.Protective foil of Determine test kitcover was removed, with the help of pipette50ul of plasma/serum was added to the sample pad marked by arrow symbol and results were read in a minimum of 15minutes and maximum of 60minutes. The results were read as either negative or positive, samples that tested positive were confirmed using Unigold test kits as guided by National serial algorithms.

ELISA technique for HTLV-1 Determination: 50µl of negative and positive controls were added to the negative and positive control wells respectively. In sample wells, 40µl Sample dilution buffer and 10µl sample were added. Samples were loaded onto the bottom without touching the well wall and mix well with gentle shaking. After 30 minutes of incubation at room temperature and sealed with closure plate membrane, diluted with buffer 30 times for 96T and washed 5 times. 50µl HRP-Conjugate reagent was added to each well except the blank control well, it was incubated for 30 minutes and washed. 50µl Chromogen Solution A and 50µl Chromogen Solution B was added to each well, mixed with gentle shaking and incubated at 37 ℃ for 15 minutes, light was avoided during colouring. Lastly, 50µl of stop solution was added to each well to terminate the reaction and colour in the well changed from blue to yellow. Absorbance was Read at 450nm using a Microtiter Plate Reader and OD value of the blank control well is set as zero.

Ethical Clearance: An ethical approval was obtained from Jigawa State Ministry of health ethical committee before the commencement of the study, and the consent/accent of the subjects was sought for during this study. The subject was giving an opportunity to accept or reject enrolment into the study.

Statistical Analysis: The data obtained were entered in Microsoft Excel, analysed and presented as numerical simple proportions and percentages using statistical software SPSS Version 20. Chi- square test was also used to compared variables using $P<0.05$ as statistically significant.

RESULTS

Table 1 shows the prevalence of HTLV-1 IgG and IgM antibodies among HIV patients enrolled in this study. 15% that is nine out of sixty tested positives to HTLV-1 IgG antibodies while 6.6% that is four out of sixty tested positives to HTLV-1 IgM antibodies. However statistical analysis shows that there is no significant difference between IgG and IgM antibodies among the HIV subjects.

Table 2 shows the evaluation of CD+4 cells counts of HIV patients and its relationship with HTLV-1 IgG and IgM antibodies. The HIV patients are grouped based on Centre for Disease control(CDC) categorization. Individuals with CD4 counts 0-200 tested negative to both IgG and IgM antibodies, those with CD4 of 200-499 has 10% and 3.3% who tested to positive HTLV-1 IgG and IgM antibodies respectively. Individuals with CD4 counts >500 has 5% and 3.3% who tested positive to HTLV-1 IgG and IgM antibodies

Table 3 shows age distribution with respect to HTLV-1 IgG and IgM antibodies among HIV subjects. Age group 5-14 and 55-64 all tested negative to both HTLV-1 IgG and IgM antibodies. Age group 15-24, 24-34, 35-44 and 45-54 has a percentage prevalence of IgG antibodies of 3.3%,5%,5% and 1.6% respectively. However, age group 25-34 is the only age group that has individuals who tested positive to HTLV-1 IgM antibodies with 6.6%.

Table 1: Prevalence of HTLV -1 IgG and IgM Antibodies Among Study Subjects

Sex	No. Screened	IgG (%)	IgM (%)
Male	20	04(6.6)	02(3.3)
Female	40	05(8.3)	02(3.3)
Total	**60**	**09(15)**	**04(6.6)**
X^2 = 0.1741		P – valve = 0.6765	

Table 2: Relationship between CD4⁺ cells counts and HTLV -1 IgG and IgM Antibodies Among HIV Subjects According to CDC Categorization.

CD4 –cells counts/µl	No. Screened	IgG %	IgM%
0 - 200	05	00(0)	00(0)
200 – 499	32	06(10)	02(3.3)
>500	23	03(5)	02(3.3)
Total	**60**	**09(15)**	**04(6.6)**

Table 3: Age distribution and HTLV -1 IgG and IgM Antibodies Among Subjects

Age Range	No Screened	IgG (%)	IgM (%)
5 – 14	03	00(0)	00(0)
15 – 24	05	02(3.3)	0(0.0)
25 – 34	24	03(5)	04(6.6)
35 – 44	20	03(5)	00(0)
45 – 54	05	01(1.6)	00(0)
55 – 64	02	00(0)	00(0)
Total	**60**	**09(15%)**	**04(6.6%)**

DISCUSSION

Human T-cell virus type 1 (HTLV-1) is the causative agent of tropical spastic paraparesis (TSP), a disease also known as HTLV-1-associated myelopathy (HAM) (Guzman and Sands, (2007) and also Adult T-cell Luekemia (ATL), the earlier is an upper motor neuron syndrome affecting lower extremities. There is contradicting evidence that HTLV-1 can remain latent for up to 20-30 years after initial infection but an average of 3years is more typical (Szczypinska *et al.*, 2014).

The highest prevalence of HTLV-1 infection has been reported in Caribbean island and south America, with moderate distribution in Japan and Africa (Proietti *et al.,* 2005). According to the results obtained from the present study, an overall prevalence of (15% for IgG and 6.6% for IgM) antibodies were recorded among HIV patients, this is in line with work of Piehl *et. al.* (1988) who reported an overwhelming 14% of HTLV-1 infection in Papua New Guinea. A study conducted by Shmuel *et al.* (2009) documented 5.8% of HTLV-1 infection among blood donors in Israel, In Nigeria a study conducted by Mohammed *et al.* (2006) recorded a lower prevalence of 3.2% among blood donors in Jos Nigeria. Similarly, it is reported that about 5-10 million individuals are infected with HTLV-1 among 1.5 billion of people living in HTLV-1 endemic areas (Gessain *et al.,* 1992). However, in Nigeria, most of the HTLV-1 seroprevalence research were mostly on blood donors. In north-western Nigeria Analo 1998) reported percentage prevalence of 3.7 in Zaria. More recently, [17] reported a prevalence of 4.9% among HIV patients attending National Hospital Abuja capital city of Nigeria.

The current finding revealed 15% prevalence of HIV co-infected with HTLV-1 (Table 1.) (Sobesky *et al.,* 2000; Goon *et al.,* 2004) both reported percentage prevalence of 9.9% and 31.8% respectively. The co-infection of HIV/HTLV-1 may be due to upward regulation of HTLV-1 by HIV which results in consequent pathological manifestation such as TSP/HAM and ATL leading to faster progression of AIDS and shorter survival rate, the existence of the same transmission route for both HIV and HTLV-1 and same tropism for T-lymphocytes may play significant role in co-infection. Irrespective of sex or baseline CD4 counts, HIV-HTLV-1 co-infected individuals are more prone to death than individuals who HIV mono infected (Brites *et al.,* 2001).

Some degree of immunosuppression caused by HTLV-1 infection has been documented by [20] resulting to other maladies such as crusted scabies, strongyloidiasis and Tuberculosis (Brites *et al.,* 2002; Porto *et al.,* 2002).

The two most indispensable tools used before commencing antiretroviral therapy (ART) in Nigeria are CD4 counts and recently viral load, but the most commonly used tool used in Nigeria in monitoring antiretroviral therapy is monitoring of patients CD4 counts. This study evaluates CD4 counts of HIV subjects in relation to HTLV-1 infection and it was found that individuals with CD4 counts 0- 200 cells/µl has zero percent prevalence of both HTLV-1 IgG and IgM antibodies, while those with CD4 counts 200-499 cells/µl have 10% and 3.3% prevalence of HTLV-1 IgG and IgM HTLV-1 antibodies respectively, HIV patients with CD4 Counts greater than 500cell/µl have 5% and 3.3%. These findings are in agreement with that of Mazanderani *et al.* (2013) who documented raised CD4 counts in HIV/HTLV-1 co-infection. HTLV-1 promotes clonal expansion of CD4-infected T-lymphocytes causing an elevation of less competent CD4+ T-cells in coinfected persons (Zane *et al.,* 2009) the higher CD4 counts may have resulted in a delay in introduction of antiretroviral therapy in these co-infected patients. Looking at the age distribution of the study individuals in the age group 25-34 and 35-44 have equal percentage prevalence of 5% for HTLV-1 IgG antibodies, age group 25-34 is the only age group with individuals who tested positive to HTLV-1 IgM antibodies with 6.6% prevalence.

CONCLUSION

Recently, Human T-lymphotrophic virus type 1 draws the attention of researchers in the 21st century because of its pathological significance. The present study evaluated HIV subjects in Dutse Metropolis for HTLV-1 IgG and IgM antibodies, of which high prevalence of 15% and 6.6%was observed respectively.

Among the subjects with CD4 counts 0-200cells/µl were tested negative to both HTLV-1 IgG and IgM antibodies while those with counts 201-499 cells/µl has 10% and 3.3%% respectively. Health care centres in Nigeria needs to include HTLV-1 diagnosis because this will go long way in creating awareness about the virus as well as reduce the chances of its spread.

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RECOMMENDATION

Human T-Lymphotrophic Virus type-1 is one of the poorly diagnosed virus in Nigeria, most of the studies conducted in Nigeria and other African countries shows that its conducted on blood transfusion subjects, however this virus needs to be diagnosed among HIV and patients in Nigeria looking at the facts that co-infection with HTLV-1 among these subject's increase immunosuppression and mortality.

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