Original Article

Seroprevalence of HTLV-I/II amongst Blood Donors in Osogbo, Nigeria.

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Abstract

Background: HTLV type I/II is a blood borne infection that can be transmitted via blood transfusion.

Objective: To determine the seroprevalence of human T – lymphotropic virus among blood donors in Osogbo, Nigeria.

Methods: Diagnosis of Human T. Lymphotropic virus antigen was carried out on 372 serum samples among blood donors who visited the blood bank/transfusion unit of Ladoke Akintola University of Technology Teaching Hospital and Our Lady of Fatima Catholic Hospital, Osogbo between January and July 2008 using Enzyme linked immunosorbent assay techniques (ELISA) as described by the manufacturer. Western blotting was used to confirm the serum reactive samples from ELISA.

Results: Out of 372 samples analyzed, 14 (3.6%) samples were found to be positive for HTLV-I/II (7 HTLV-I and 7 HTLV-II) while 358 (96.4%) samples were negative after confirmation with Western blotting. The seroprevalence of HTLV-I/II among the blood donors in Osogbo, Nigeria was found to be 3.6%. This has major implication for the blood transfusion service in Nigeria.

Conclusion: The study concluded that there is need for screening of blood donor for HTLV-I/II in order to rule out this transfusion related infection.

Keywords: human T-lymphotropic virus, T-cell leukaemia, myelopathy/tropical spastic paraparesis.

HUMAN T-cell lymphotropic virus type 1 (HTLV-I) is a known aetiological agent of two major diseases - adult T-cell leukaemia (ATL) [1] and HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). The clinical features associated with HAM/TSP are muscle weakness in the legs, hyperreflexia, clonus, extensor plantar responses, sensory disturbances, urinary incontinence, impotence, and low back pain [2]. HTLV-II has been associated with lymphoma and cases of atypical hairy cell leukaemia but not with neurological disease. The wide spectrum of diseases associated with these viruses require that attention needs to be paid to these agents and further work is needed to determine the full public health impact of HTLV infection in the general population.

HTLV-I and HTLV-II are retroviruses members of the *Oncovirinae* subfamily. The viruses are closely related to human immunodeficiency viruses types I and II (HIV-1 and HIV-2) which belong to *Lentivirinae*. Once a cell is infected, the RNA genome is converted by reverse transcriptase to DNA and integrates into the host genome - a feature shares by HIV. HTLV-I and HTLV-II have similar genomic organization: *gag*, *pro/pol*, *env*, and *pX* flanked by long terminal repeats (LTRs) [3]. The lack of genetic variability of HTLV-I and HTLV-II, despite the long period of latency and high proviral load, is attributed to clonal expansion of HTLV-harbouring cells and not to direct viral replication, as is the case with HIV-1 and HIV-2 [4].

HTLV-I infects 15 million to 20 million people worldwide, with endemic foci in southern Japan, the Caribbean, Melanesia,

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sub-Saharan Africa, and Central and South America [5, 6]. The seroprevalence rate ranges from 3 to 6% in the Caribbean islands [7] to about 27% in southern Japan (Mueller et al., 1996). HTLV-II is endemic in Amerindian tribes throughout North and South America and in Pygmy tribes in Central Africa, with seroprevalence ranging from 3 to 33% [8, 9]. HTLV-I/II infections are contracted through sexual, vertical, and parenteral transmission. Intravenous drug abuse and sex with intravenous drug users have been found to be the most important risk factors for HTLV-II transmission [10]. The high risk of blood transfusion transmitted HTLV-I/II has prompted Japan, USA, several Caribbean countries, Canada, France, Holland, Denmark, Sweden, Portugal, Australia, and Greece [11-13]. Screening programme has been shown to reduce risk of transfusion related transmission in USA [12, 14]. It was in view of this, that this study was undertaken. Therefore the aim of this study was to determine the seroprevalence of HTLV1/2 among the blood donors in Osogbo, Nigeria in order to highlight the importance of including this in transfusion microbiology services in Nigeria.

Materials and Methods

Sample Collection and Study Site: The study was carried out at Department of Biomedical Sciences, College of Health Sciences, Osogbo after collection of 5 ml of blood from 364 prospective blood donors that came in to donate blood at Blood Bank of Ladoke Akintola University of Technology Teaching Hospital, Osogbo and Our Lady of Fatima Catholic Hospital, Osogbo between January and July, 2008. The donors were asked to fill in a standard questionnaire. Serum was separated from the blood after the blood was allowed to clot. The serum samples were stored at -20^{o}C until the assay for presence of antibodies to HTLVI/II was carried out.

Assay for Presence of HTLV1/II Antibodies: Antibodies to HTLV1/2 were assayed in serum samples in triplicates by using Vitronostika HTLV-I/II microelisa kit (Biomèrieux, France). This assay was based on the principle of "sandwich" ELISA in which the wells of the microtitre plate were coated with purified HTLV-I antigens, purified HTLV-II antigens, and a recombinant HTLV-I p21E antigen. The procedures for the assay of the presence of antibodies to HTLV-I/II were carried out as instructed by the manufacturer. Briefly, 20 µl of serum was added to each well, after which 80 µl of sample diluent provided was added, after which the mixture was incubated at 37^{o}C for 1 h to allow for antigen-antibody complex formation. The unbound antibodies were washed off the well with wash buffer (3-cyclohexil-amino-1-propane-sulphonic acid) thereafter; working solution of horse radish peroxidase labelled goat anti-human immunoglobulin solution was added to each well and incubated at 37^{o}C for 1 h. After incubation, the well was washed several times with washing buffer before the addition of tetramethyl benzidine (TMB) substrate solution. The colour was allowed to develop by incubating the mixture at room temperature for 30 min. The reaction was stopped by adding 100 µl of 1 M sulphuric acid to each well. Plates were read within 15 min; the reader was blanked against air and the absorbance of the solution in each well was read at 450 nm. Results were interpreted according to manufacturer's instructions.

Western blotting: The positive and/or borderline samples were further tested by a confirmatory Western blot assay (WB; HTLV-I/II Blot 2.4; Diagnostic Biotechnology, Singapore). A sample was considered HTLV-I positive if it reacted to the two Gag proteins (p19 and p24) and both *env*-encoded glycoproteins: the HTLV-I-specific recombinant gp46-I peptide (MTA-1) and the specific HTLV-I/HTLV-II recombinant GD21 protein. It was considered HTLV-II positive if it reacted to the Gag protein p24 and both *env*-encoded glycoproteins: the HTLV-II-specific recombinant gp46-II peptide (K55) and the GD21 protein. Plasma samples were considered negative when they exhibited no bands and indeterminate when they were partially reactive.

Data analysis: Statistical analysis was carried on the data obtained using the statistical package within Epi-info 6 developed at Centre for Diseases Control and Prevention, Atlanta, USA [15].

Results

Three hundred and seventy two samples were tested for HTLV-I/II, 14 were found to be reactive, and the percentage of reactive samples was found to be 3.8%. None of the female samples was found to be reactive to HTLV-I/II antigens (Table 1).

Table1. Prevalence of HTLV-I/II among the male and female blood donors in Osogbo

Sex	Number	+ve (%)	-Ve (%)
Male	332	14 (4.2)	318 (89.8)
Female	40	0 (0)	40 (10.2)
Total	372	14 (3.8)	358 (96.2)

On confirmation with Western blotting following the manufacturer's interpretation, seven of the 14 samples were found to be reactive with HTLV-I antigens (p19, p24, and MTA-1) while the remaining seven were found to be reactive with HTLV-II antigens (p24, K55, and GD21). There was no significant difference between the frequency of seropositivity between male and female subjects ($X^2 = 0.78$; $p > 0.05$).

Among the male subjects, 14 were found to be reactive to HTLV-I/II antigens representing 4.2% of the total male subjects in this study. The result also showed that the proportion of seropositivity was high in frequent donors (20%, odds ratio (OR) = 6.83) compared to blood donors who had donated between 1 and 3 times (2.6 – 3.5%) (Table 2).

Table 2: Comparison of HTLV-I/II positive people with number of donations

No of donations	Number of Subjects	Number Reactive to HTLV 1/2	% HTLV 1/2 Seropositivity.	Odds Ratio
1	170	6	3.5	1.00
2	78	2	2.6	0.72
3	114	4	3.5	0.98
>3	10	2	20	6.83

Table 3: The effect of exposure to blood on blood donors on HTLV-I/II reactivity.

Exposure to blood	Number of Subjects	HTLV-I/II Reactive
Yes	52	1 (3.85%)
No	310	6 (3.87%)
Undetermined	10	0 (0%)

Number of subjects that were seropositive to HTLV-I/II antigen was found to be comparable between subjects that had been previously exposed themselves to blood products and those subjects that had not been exposed to blood or blood products (Table 3). The prevalence of HTLV-I/II infections was confined to two different age groups – age groups between 18 and 24 (5.9%) and 25 and 31 (12.5%) with other different age groups showing zero prevalence (Table 4). There was statistical significant difference when age groups between 18 and 31 were compared with other different age groups – 32 and above ($X^2 = 19.48$, $p < 0.05$). Although the commercial donors were not represented in this study, this study found there were high proportions of reactivity to HTLV-I/II antigens among family (3.2%) and voluntary (6.7%) blood donors (Table 5). No significant difference was found between the frequency of seropositivity among family and voluntary donors (X^2 test, p value > 0.05) The distribution of seropositivity among different occupations showed that the drivers recorded highest seroprevalence (18.2%) to HTLV-I/II as indicated on Table 6 with lowest recorded among unemployed.

Table 4: Age Distribution of HTLV Seropositivity among blood donors in Osogbo

Age Group	Number of Subjects Tested	Number of Samples Reactive to HTLV-I/II	% HTLV-I/II seropositivity
18-24	68	4	5.88
25-31	80	10	12.5
32-38	140	0	0
39-45	56	0	0
49-52	16	0	0
53-62	12	0	0

Table 5: HTLV-I/II with Donor type

Type of Donor	Number Tested	HTLV 1/2 Reactive	Percentage Reactivity
Family	312	10	3.2
Voluntary	60	4	6.7
Commercial	0	0	0

Table 6: Occupation of the donor with HTLV

Occupation	Number of donor	HTLV-I/II Reactive	% Reactivity. HTLV-I/II	Odds Ratio(OR)
Driver	22	4	18.2	1.00
Business	24	2	8.3	0.41
Student	40	4	10	0.50
Security	10	0	0	0.00
Civil servant	140	2	1.4	0.07
Unemployed	40	2	5.0	0.24
Others	96	0	0	0.00

Discussion

HTLV-I/II prevalence has been reported from different countries all over the world. For example, USA has reported 3-5% prevalence [16]; in Japan where the HTLV-I/II has been shown to be endemic, the prevalence was put at 27% [17]; while the prevalence in Central Africa ranges from 3 to 33% [8, 9]. Study carried out among the commercial sex workers and pregnant women in south western Nigeria recorded seroprevalence of 22.9% and 16.7%, respectively [18]. Recent work carried out in

Dakar, Senegal showed the prevalence of HTLV-I/II among blood donors was put at 0.16% [19] as opposed to study carried out in 1986 in Nigeria that found seroprevalence to be 2.0% [20]. In this study, the seroprevalence of HTLV-I/II was carried out in order to determine whether there is any need to start screening blood donors for these infectious agents – HTLV-I/II. This study was carried out using Vironostika HTLV-I/II developed by Biomerieux which the manufacturer claimed to have 100% sensitivity and specificity for screening and used Western blotting to confirm the reactivity of the samples found reactive in ELISA. In low resource countries like Nigeria, the ELISA can be used for screening of blood donors and any reactive sample can be prevented from being used for blood transfusion. Also, we found no evidence of cross reactivity with HIV as all the donors that were reactive to HTLV-I/II antigens were found

not to be reactive to HIV antigen. Cross reactivities between HTLV and HIV has been reported before by Olaleye *et al* [21]. In this study, the prevalence of HTLV-I/II was found to be 3.8% among the blood donors in Osogbo, Nigeria. This result has contradicted the result obtained in a similar study carried out among blood donors in nearby West African country – Senegal, where they found it to be 0.16% [19] but it is in conformity with a study carried out in Caribbean countries where they found the prevalence to be 3-6% [7].
It is very interesting to note that the seroprevalence of HTLV-I/II among older age groups (32 years and above) in this study was zero while the seroprevalence among younger age groups (age group between 18 and 31 years) was 9.45%, statistically significantly higher than other age groups (p value < 0.05), suggesting that this infection was not endemic in the past in part of Nigeria and it is now in the community as a result of a new way of life among this age group. This study was unable to identify the risk factor that predisposes this particular age group to HTLV-I/II infection so that necessary intervention can be introduced to control the infection. A study carried out in south western of Nigeria found the prevalence to be between 16 and 23% among the sexually active individuals including commercial sex workers [18]; it is possible to attribute this factor to the high prevalence of HTLV-I/II in this age group.
Furthermore, previous exposure to blood or blood products is shown not to play any significant role in the transmission of the microbe in this study, indicating transmission of the infection is not transfusion related only. A recent study carried on commercial sex workers found the prevalence to be 23% [18]. Motor vehicle driver as an occupation has been pinpointed as occupation associated with this high prevalence of HTLV-I/II infections. This occupation has not been associated in coming in contact with blood or blood products. The plausible explanation that could be given for this is the ability of this group of professionals (drivers) to travel from home, and as a result of that they derive sexual pleasures from prostitutes at their temporary destination. Alternative reason is also they are group of individuals that are prone to motor accident as a result of their daily activities. Some of these accidents might require them of taken blood as a result of severe blood loss due to injury, subsequently leading to the risk of been infected with HTLV. Seropositivity of 4.8% was found among blood donors that have donated blood 3 times or more in this study, considering the fact that we have no commercial donor in this study (Tables 2 and 5).
HTLV infection has been shown to be transfusion related infection[22]. In fact, posttransfusion infections due to HTLV have been reported[22-24]. Many countries in the world where HTLV is endemic and other countries with low prevalence have taken steps to control this infection. One of such intervention is to screen blood donor(s) for evidence of acquiring HTLV in order to prevent transfusion related infection. A similar measure has been taken to arrest the spread of HIV through blood transfusion. This important intervention has been shown to reduce the transmission rate of HTLV in USA [12, 14]. Studies carried out in the 1980s showed the presence of antibodies to HTLV among blood donors in Nigeria [20, 25], despite this data, HTLV screening is yet to be incorporated into screening of blood donors in Nigeria and many Africa countries.

Since this organism can be incorporated into host genome in form of proviral DNA like HIV, it is as good as saying this is a lifetime infection with no hope at the moment of successful treatment. Hence, subjects that are seropositive for HTLV are reservoir of infection for HTLV. Screening of blood donors for antibodies will eliminate the use of blood from subjects that have acquired HTLV infection. HTLV-I/II infection has increased from 2.0% [20] to almost 4.0% in this survey – a modest increase, this study strongly advocates for screening of blood donors for HTLV because the level of infection is high enough to start including HTLV screening as part of blood transfusion microbiology services in all the blood banks laboratories.

Conclusion:

The study concluded that the prevalence of HTLV infection is high enough to warrant the screening of blood donors for exposure to HTLV.

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