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Seroprevalence of Human T Cell Lymphotropic Virus I/II among Blood Donors in Sokoto Nigeria.

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Abstract

HTLV is blood borne infectious agents that can be transmitted via blood transfusion. This study was carried out to determine the seroprevalence of Human T-lymphotropic virus infection among blood donors in Sokoto Northwestern Nigeria. Diagnosis of Human T-Lymphotropic virus antigens was carried out on serum samples of 100 consecutively recruited blood donors who visited the blood bank of Usmanu Danfodiyo University Teaching Hospital and Sokoto Specialist Hospital for blood donation purpose. The blood donors were aged 18-65 years with mean age of 30.68±7.09 years and made up of 95 (95%) males and 5 (5%) females. Among the donors tested 1(1%) was positive while 99 (99%) were negative for HTLV. HTLV prevalence was only observed among male blood donors, 1(1.1%) while 94 (98.9%) were negative for HTLV (p -value = 0.818). Also HTLV was higher among blood donors aged between 31 to 35 years, (p -value = 0.814). HTLV prevalence was compared based on the occupational group of blood donors. HTLV prevalence was also higher among civil servant 1 (4.3%) (p -value= 0.496). The prevalence was compared based on ethnicity most of the subjects 76 (76%) were Hausas. Of this number 1 (1.3%) was positive for HTLV (p -value= 0.956). HTLV prevalence was also observed among donors with history of high risk behaviors 1(1%) (p -value=0.685). No seropositivity was observed among those who donated blood in the past and those with history of previous transfusion. The seroprevalence of HTLV is low in this environment. Therefore large prospective study is required to establish true seroprevalence of HTLV among blood donors in Sokoto Northwestern Nigeria.

Introduction

The human T-lymphotropic virus (HTLV) was the first human retrovirus discovered (Gallo, 2005). In 1979, T-cell lymphotropic virus was isolated in a

patient with cutaneous T-cell lymphoma (Poiesz *et al.*, 1980). This led to the discovery of the first human T-lymphotropic virus and marked the beginning of the human retrovirus era.

Human T-lymphotropic viruses (HTLV) belong to Retroviridae family, genus Delta retrovirus. Currently they are classified into four types: 1, 2, 3 and 4. The HTLV-1 was described in 1980 (Poiesz *et al.*, 1980) and since then, the virus has been identified on all five continents, with an estimation of 15 to 20 million infected people (Gessain *et al.*, 1996). The areas are characterized as endemic, where the prevalence ranging from 0.5 to 20% in the population, depending on age and gender, or not endemic, where the prevalence is less than 0.1%. The seroprevalence rates increase with age and are higher in females than males

HTLV infections are more prevalent in Japan, sub-Saharan Africa, and Caribbean basin, South America, Melanesia and the Middle East. The HTLV-2 was described in 1982 (Kalyanaraman *et al.*, 1982) and it is endemic in African and Amerindian populations, but its worldwide distribution has been ascribed to transmission among intravenous drug users.

HTLV-3 and 4 were discovered in a rural area of Southern Cameroon (Mahieux and Gessain, 2011; Wolfe *et al.*, 2005) and, at present, they are restricted to that region. HTLV-1 and 2 are transmitted sexually and vertically, firstly by breastfeeding, as well as parentally, by contaminated blood transfusion, sharing of contaminated needles and syringes, or transplantation of infected organs and tissues. Seropositive mothers acquire HTLV infection through blood transfusion. Different

countries have introduced screening for the viruses in blood banks, some of them across the country and others only in endemic areas. Thus, HTLV-1/2 screening of blood units is important to prevent the most cases of transfusion transmitted infection, but the relatively long HTLV immunological window period (51 days) may lead to its transmission (Manns *et al.*, 1992). This is the reason why it has become necessary to establish haemovigilance actions in blood banks.

Material and Method

Study Area

The selected area for this study is Usmanu Danfodiyo University Teaching Hospital (UDUTH) which is located in Wamakko Local Government and Specialist Hospital within Sokoto Metropolitan city in Sokoto State. Sokoto State is located at the extreme Northwest of Nigeria Near the confluence of the Sokoto River and Rima River, with an annual average temperature of 28.3°C (82.9 °F). Sokoto is On the whole, a very hot area, Maximum day time temperatures are for most of the year generally under 40°C (104.0 °F). The warmest months are February to April when daytime temperatures can exceed 45°C (113.0 °F). The rainy season is from May to October during which showers are a daily occurrence. There are two major seasons, wet and dry which are distinct and are characterized by high and low malarial transmission, based on 2006 population census (NPC, 2006). Sokoto State had a population of 3,696,999 (Sokoto Diary, 2008), with an average estimate of 4,806,098 in 2015 based on the population annual growth rate of 3% (NPC, 2006). This study was carried out in UDUTH Sokoto. The teaching hospital is based in Sokoto town, Sokoto state and was established in May 1980 as a second generation teaching hospital along with Port-Harcourt, Ilorin, Calabar, Jos, and Maiduguri Teaching Hospital. The teaching hospital provides high quality tertiary health care services to the entire North-Western region of Nigeria and neighboring Niger Republic.

Inclusion criteria

Inclusion criteria included; Age (18-65 years), No history of blood transfusion within the last 3 months, willingness to give written informed consent after counseling and non-menstruating women .

Exclusion criteria

All consecutively recruited blood donors who did not meet the inclusion criteria were not included in

this study; Age (<18 years, >65 years), history of blood transfusion in the last 4 months, unwillingness to give written informed consent and menstruating women will be excluded from this study. .

Sample collection

Three milliliters (3.0ml) of venous blood samples was collected from each donor into a clean dry tube and allowed to stand for 30 minutes at room temperature for clotting and retraction. The samples were centrifuge at 3000rpm for 10 minutes; the serum was separated and analyzed immediately. The manufacturer's standard operating procedure was followed strictly

HTLV Screening

The kit is an antigen "sandwich" enzyme immunoassay (ELISA) method, which uses polystyrene microwell strips pre-coated with recombinant HTLV antigen, It is a one step incubation. The serum/plasma sample is incubated in the microwell together with second recombinant HTLV antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. The specific antigen-antibody immunocomplex is captured on the solid phase. After washing to remove sample and unbound HRP-Conjugate, Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-antibody-antigen (HRP) "sandwich" complex, the colorless Chromogen are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The sulfuric acid stops the reaction, and then the blue color turns yellow. The amount of color intensity is measured. It is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HTLV remain colorless.

Procedure

The standard was added in to the micro Elisa strip plate and 50 µl of positive and negative control was added separately to the positive and negative well, 50 µL of sample was added to the testing sample well, 100µL of HPR-conjugate reagents was added to each well and covered with adhesive strip and incubated for 60 minutes at 37°C, after incubation each well was aspirate and wash for maximum of five washes, 50 µL of Chromogen solution A and Chromogen solution B was added to each well; it was mixed gently and incubated for 15 minutes at 37°C, 50 µL of Stopping Solution was added into

each well and mixed gently, intensive yellow color was observed in positive control and HTLV positive sample wells, the optical density (O.D) of each sample was read at 450nm using a microtiter plate reader within 15 minutes and the cut-off value was calculated and the results were evaluated.

Data Analysis

Data collected was analyzed using SPSS Version 20. Data collected was descriptive. Results was expressed as mean and standard deviation. Differences in values based on socio-demographic variables of subjects will be determine and compared statistically using chi-square. A p-value of ≤ 0.05 was considered as significant in all statistical comparisons.

Results

A total of 100 consecutively recruited blood donors were enrolled into this study. Majority of them were aged less than 35 years 86 (86%) with a mean age of 30.68 ± 7.09 years. Among the donors tested 1(1%) was positive while 99 (99%) were negative for HTLV. HTLV was compared on based marital

status. Table 1 shows the HTLV distribution based on age groups. Out of 100 blood donors tested, majority of them were married 50 (50%) compared to single 47(47%) and had secondary school education or less. Out of 47 single blood donors 1(2.1%) was positive for HTLV (p-value = 0.566). Table 2 shows the HTLV distribution based on marital status. The distribution was also compared among ethnic groups; most of the subjects 76 (76%) were Hausas. Of this number 1 (1.3%) was positive for HTLV (p-value= 0.956). Table 3 shows the HTLV distribution base on ethnic groups. HTLV was compared based on occupation, majority of the participants 77 (77%) were either civil servants, business men and unemployed. Out of 23 civil servant 1 (4.3%) was positive for HTLV (p-value= 0.496). Table 4 shows the HTLV distribution based on occupation. HTLV was insignificant in this study based on risk behaviors, among donors tested 1(1%) positive for HTLV (p-value=0.685). Table 4 shows the HTLV distribution based on high risk behaviour among blood donors.

Table 1:HTLV distribution based on Age groups

Age groups (Years)	No.(%) of donor tested	HTLV (%)	
		Positive	Negative
18-25	19	0	100
26-30	36	0	100
31-35	31	3.2	96.8
36-40	10	0	100
41-45	1	0	100
46-50	3	0	100
Total	100	1	99
Chi-square			2.248
P-value			0.814

Table 2: HTLV distribution base on marital status

Marital status	No. (%) of donor tested	HTLV (%)	
		Positive	Negative
Married	50	0	100
Single	47	2.1	97.9
Separated	3	0	100
Divorced	0	0	100
Widowed	0	0	100
Total	100	1	99
Chi-square			1.139
p-value			0.566

Table 3 HTLV distribution base on ethnic groups

Ethnic groups	No. (%) of donor tested	HTLV (%)	
		Positive	Negative
Hausa	76	1.3	98.7
Yoruba	10	0	100
Igbo	7	0	100
Others	7	0	100
Total	100	1	99
Chi-square			0.319
p-value			0.956

Table 4: HTLV distribution based on occupation

Occupation	No. (%) of donor tested	HTLV (%)	
		Positive	Negative
Civil servant	23	4.3	95.7
Farming	7	0	100
Business	34	0	100
Unemployed	20	0	100
Student	16	0	100
Total	100	1	99
Chi-square			3.382
P-value			0.496

Table 5: HTLV distribution base on risk behaviors

Risk behaviors	No. (%) of donor tested	HTLV (%)	
		Positive	Negative
Unprotected sex			
Yes	3	0	100
No	97	1	99
Sharing needle/sharp objects			
Yes	14	0	100
No	86	1.1	98.8
Total	100	1	99
Chi-square			0.164
p-value			0.685

Discussion

HTLV prevalence has been reported from different countries all over the world. For example HTLV prevalence has been reported in USA at 3-5% (Manns *et al.*, 1999); in Japan where the HTLV has been shown to be endemic, the prevalence was put at 27% (Mueller *et al.*, 1996); while the prevalence in Central Africa ranges from 3 to 33% (Hjelle *et al.*, 1994). Recent work carried out in Dakar, Senegal showed the prevalence of HTLV among blood donors was put at 0.16% (Diop *et al.*, 2006) as opposed to study carried out in 1986 in Nigeria that found seroprevalence to be 2.0% (Fleming *et al.*, 1986).

This study was carried out to determine the seroprevalence of HTLV among blood donors in Sokoto Northwestern Nigeria. Our observed a HTLV prevalence of 1%, is in conformity with

previous reports in Calabar (Fleming *et al.*, 1986), Enugu (Okoye *et al.*, 2015) and Lagos South-western Nigeria which reported a prevalence of 0.7%, 0% and 0.7% respectively.

This study was carried out using HTLV ELISA Kit developed by BIOTAIN China. The manufacturer claims to have 100% sensitivity and specificity for screening 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. In this study, the prevalence of HTLV was found to be 1% among the blood donors in Sokoto Northwestern 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% (Dioup *et al.*, 2006). Furthermore, previous exposure to blood or blood products is shown not to play any significant role in

the transmission of the virus in this study, indicating that transmission of the infection is not transfusion related. HTLV infection has been shown to be transfusion-transmissible infection; However post transfusion infections due to HTLV have been reported (Kaplan *et al.*, 1991). 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-transmissible 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 (Schreiber *et al.*, 1997). Studies carried out in the 1980s showed the presence of antibodies to HTLV among blood donors in Nigeria (Okpara *et al.*, 1988) 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 information of proviral DNA like HIV, it is as good as saying that HTLV 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 infections. This study strongly advocates for screening of blood donors for HTLV because the level of infections are high enough to start including HTLV screening as part of blood transfusion services in all the blood banks laboratories in Nigeria like other transfusion transmissible infections .

Conclusion and Recommendation

The seroprevalence of HTLV infections among blood donors who participated in this study was found to be 1%. The low prevalence obtained for HTLV in this study made it statistically difficult to establish a correlation between the effect of various socio-demographic variables and the prevalence of HTLV infections. Prospective and multicentre research on the seroprevalence of HTLV infection among blood donors should be encouraged to help to justify the routine screening of HTLV infection for blood donors. A standard and simultaneous national epidemiological survey should be carried out in all the geopolitical zones of Nigeria to establish the true seroprevalence of HTLV and their pattern of distribution.

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