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Serosurvey of human T cell lymphotropic virus I/II among blood donors in Gombe (Nigeria)

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ABSTRACT

Aim: This study was carried out to determine the prevalence of human T cell lymphotropic virus I/II (HTLV I/II) among blood donors at the Federal Teaching Hospital Gombe (FTHG), North-eastern, Nigeria. Methods: A total of 355 blood donors were recruited at the hematology department of FTHG for a hospital based cross-sectional study and were screened/confirmed for HTLV I/II by ELISA and western blot methods. Results: Seroprevalence of HTLV I/II among the 355 participants with mean age of 30.77 years (±8.27) was 6.5% on screening with ELISA but confirmed to be 0% by western blot. Majority were males, 352 (99.2%) and mostly (45.9%) between 21–30 years of age. Donors for family replacements predominated (85.1%) with more than half (58.6%) being first time blood donors. Conclusion: This study reveals the absence of HTLV I/II among blood donors in Gombe North-eastern Nigeria. It also highlights the possibility of false positivity in some of the reported prevalence of HTLV I and II from some parts of Nigeria that were based on screening tests only. The importance of confirmatory testing in all research works on HTLV (including HTLV 3 and HTLV 4) is further strengthened with this study. There is however the need for a larger study and the use of molecular diagnostics to reconfirm this assertion.

Keywords: Blood donors, Gombe, Human T cell lymphotropic virus I/II (HTLV I/II), Nigeria, T cell

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INTRODUCTION

Human T cell lymphotropic virus I (HTLV-I) is the first human retrovirus to be discovered and has continued to be an important transfusion transmissible infection (TTI) especially in highly endemic regions such as the sub-Saharan Africa [1]. The virus has been associated with several diseases including adult T cell leukemia (ATL), tropical spastic paraparesis, HTLV-I uveitis and HTLV-I associated infective dermatitis [2, 3]. The ATL develops only in HTLV-I infected individuals and all ATL cells contain integrated HTLV-I provirus [4]. Geographical areas with high incidence of ATL patients correspond closely with those of high incidence of HTLV-I carriers [5]. However, HTLV-II has not been causally linked to human malignancy but may be associated with lymphocytosis, increased risk of developing inflammatory neuropathies and infectious diseases [6].

Blood safety remains an important public health concern in Africa as morbidity and mortality from unsafe blood continues to adversely impact on populations [7]. Human T cell lymphotropic virus has been considered to be currently one of the greatest concerns threatening health of blood recipients in some endemic areas [8]. Intravenous exposure to contaminated blood or blood products is the most efficient mode of HTLV-I transmission with up to 63.4% seroconversion rate reported in Japan [3, 9]. Additionally, blood transfusion related HTLV associated diseases develop more rapidly than with other means of transmission [10].

Immunosuppression increases the risk of HTLV transmission with shorter latency period when compared to healthy carriers [11]. The HIV pandemic has led to an upsurge in the number of immunosuppressed patients, thus supporting the need for pre-transfusion screening for TTIs including HTLV. This is more important in highly endemic regions, including Nigeria. HTLV-I/HIV-1 co-infection is associated with severe clinical manifestations of HIV and worsening of the immunosuppressive status of the patient [12]. In Africa, this becomes a major concern due to the high burden of HIV infection in the region [13].

Routine screening for antibodies to HTLV-I and HTLV-II has been instituted in blood banks in many countries including Japan, USA, Canada, the Caribbean, France, Brazil and Australia [2, 3]. Presently in African countries Nigeria inclusive, there is no routine screening programme for HTLV among blood donors [14, 15]. Prevalence studies on HTLV from some regions of Nigeria do exist. However, there is paucity of data on the burden of this virus from other parts of Nigeria especially the north-eastern region.

This current discourse is aimed at establishing the prevalence of HTLV I/II among blood donors in Gombe, Nigeria.

MATERIALS AND METHODS

STUDY POPULATION

Healthy blood donors presenting at the blood bank unit of the Haematology department Federal Teaching Hospital Gombe (FTHG) who met the inclusion criteria were recruited after written informed consent was sought and obtained. The inclusion criterion was: (i) all consenting apparently healthy blood donors between the ages of 18 and 65 years. The exclusion criteria were: (i) refusal to give consent for participation in the study, (ii) individuals not eligible to donate blood. (Supplementary Table 1: Criteria for eligibility to donate blood. Available online at the journal's website).

Ethical clearance and approval was obtained from the research and ethics committee of the FTHG for the study. Handling, storage and disposal of the specimens was strictly based on sound ethical principles.

STUDY DESIGN

This hospital-based cross-sectional study was done using enzyme linked immunosorbent assay (ELISA) and Western Blot (WB) to detect and confirm HTLV I/II among apparently healthy blood donors.

Sample size was determined using the Fisher's formula as 333 based on the HTLV prevalence of 3.6% [16] among blood-donors from northern Nigeria. This was readjusted to 366 by adding 10% of the calculated figure to make room for attrition. Three hundred and sixty-six donors were recruited for the study but 355 were analysed as 11 specimens were found to be unsuitable for the study. A semi-structured questionnaire was administered to each participant.

LABORATORY METHODS

Specimen collection and storage

Five milliliter of venous blood was collected aseptically from all consenting participants into an EDTA specimen container with immediate separation of plasma from other components. The plasma component was then stored at –20°C.

Screening and confirmation

HTLV I/II screening was done using HTLV I & II Ab version ULTRA EIA assay kit by DIA.PRO diagnostic bioprobes Milano Italy [17]. All positive samples from the ELISA were confirmed using WB with HTLV BLOT Version 2.4 by MP Biomedicals Asia Pacific Pte. Ltd. (formerly Genelabs Diagnostics Pte. Ltd.) Singapore [18].

All tests and result interpretations were done following the manufacturers' guidelines.

DATA ANALYSIS

Descriptive and inferential statistical data analysis was done using Statistical package for the social sciences version 22 (SPSS Inc., Illinois, USA).

RESULTS

Age and sex distribution of the participants

A total of 366 blood donors were recruited but 355 were studied. Their age range was 18 to 58 years with a mean of 30.77 (±8.27) years. Donors between 21 and 30 years of age constituted the majority with 45.9% (163) while those above 40 years with 12.7% (45) were the least among the study population, (Figure 1). Male donors were predominant as they accounted for 99.2% (352) of the respondents with females constituting only 0.8% (3).

Behavioral characteristics of the blood donors

Behavioral characteristics of the donors are presented in Table 1. Virtually all (99.7%) the respondents never had any blood transfusion as only one (0.3%) had a history of blood transfusion. Most of the blood donors (86.4%) reported not having multiple sexual partners and only 48 (13.6%) had more than one sexual partner. First time blood donors were more and constituted 58.6% (208) of the respondents as against 147 (41.4%) who had history of prior blood donation. Majority of the donations were made for family replacement purposes (85.1%), followed by voluntary donations (14.6%), while donations for commercial purposes were the least frequent at 0.3%.

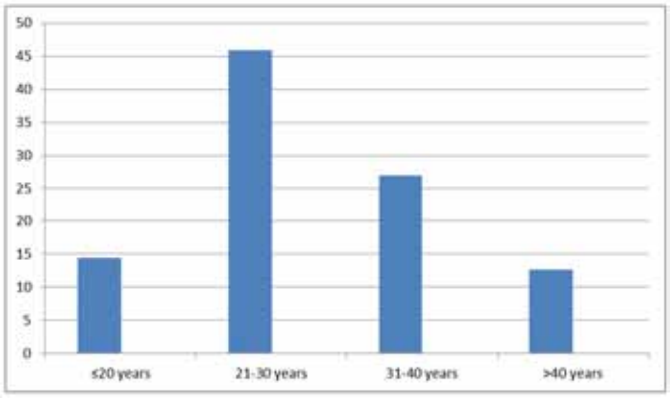

Figure 1: Percentage proportion of different age groups among the blood donors
Age groups ≤ 20 years, 21–30 years, 31–40 years and >40 years constituted 14.4%, 45.9%, 27.0% and 12.7% respectively.

Prevalence of HTLV I and II among the blood donors

The prevalence of HTLV I and II based on ELISA test alone was 6.5% (n=23). None of the respondents had an indeterminate result and all the positive specimens from ELISA were confirmed negative by western blot (Table 2).

Table 1: Behavioral characteristics of the blood donors

Variable	Frequency	%
Multiple sexual partners		
Yes	48	13.6
No	306	86.4
Number of multiple sexual partners		
Two	36	75.0
Three	09	18.8
More than three	03	6.3
History of blood transfusion		
Yes	01	0.3
No	354	99.7
Previous blood donation		
Yes	147	41.4
No	208	58.6
Number of previous blood donations		
One	97	66.0
Two	19	12.9
Three	09	6.1
Four	04	2.7
More than four	18	12.2
Purpose of donation		
Voluntary	52	14.6
Family replacement	302	85.1
Commercial	01	0.3
Drug Abuse		
Yes	03	0.8
No	352	99.2
Total	355	100

Table 2: Prevalence of HTLV I/II among the blood donors

Variable	Frequency	%
HTLV I/II (ELISA)		
Positive	23	6.5
Negative	331	93.5
Indeterminate	00	00
HTLV I & II (WESTERN BLOT)		
Positive	00	0.0
Negative	23	100
Indeterminate	00	00
Total	355	100

Abbreviations: HTLV Human T cell Lymphotropic Virus, ELISA Enzyme Linked Immunosorbent Assay

DISCUSSION

This study is about the first to be reported from any part of North-eastern Nigeria. Three hundred and fifty-five (355) blood donors in FTHG were studied and a seroprevalence of 6.5% was obtained based on ELISA which was confirmed to be 0% following western blot testing.

A recent study from Enugu in South-eastern Nigeria also reported a similar zero (0%) prevalence among prospective blood donors [19]. Some other Nigerian studies have also reported low prevalence of HTLV among different population groups [20, 21]. Low prevalence rates have also been reported from some African countries with 0.16% in Dakar, Senegal and 2.3% in Guinea Bissau [22, 23]. Although more than half of the studies on HTLV from Nigeria did not perform any confirmatory test, higher prevalence rates among certain high risk groups in Nigeria are not unusual [14, 24]. Geographical variations in prevalence of HTLV based on ethnic/socio-cultural and environmental differences from different parts of the world has been well documented [1]. Nigeria is both geographically and socio-culturally diverse. It is therefore not surprising to find 0% prevalence from a region of Nigeria that has hitherto never reported any study on HTLV or HTLV-associated diseases.

False positive results with enzyme immunoassays (EIAs) for antibodies to HTLV-I/II have led to the unnecessary deferral of tens of thousands of potential blood donors even in developed countries [25]. HTLV ELISA has shown very high (60–80% among blood donors) false-positive rates in areas of low prevalence and only confirmatory testing rules out actual HTLV infection [26]. However, virtually no confirmatory testing has ever been reported from northern Nigeria especially the North-eastern region. High level of false positive ELISA for HTLV from some parts of the world (e.g., 29.2% in Caracas [27] and 100% in Turkey [28]) could indicate that carrying out this study on larger sample size may reveal a similar prevalence from north-eastern Nigeria.

Cross-reactivity with *plasmodium falciparum* is one of the most suggested possible explanations for false positive HTLV ELISA especially in malaria endemic areas (like Nigeria) [29, 30]. False-positive HTLV EIA results with negative confirmatory Western blot test results have also been associated with many factors, such as influenza vaccine, severe acute respiratory syndrome, some bacterial infections, autoimmune disorders and multiple pregnancies [31–33]. Srivastava et al. demonstrated that both ELISA and Western blot assay have limitations for HTLV-I antibody detection in an ATL non-endemic population based on the many false positives obtained by ELISA, and weak/indeterminate reaction (mostly p19 band) on Western blotting [34]. Although none was observed in this study, indeterminate results had been reported to be a source of false positive ELISA tests [14, 34].

HTLV-3 and HTLV-4 which share similar genomic organization to HTLV-I and HTLV-II, and weak but reproducible cross-reactivity with indeterminate results in western blot may be a source of false positive results in serologic assays employing HTLV-I and HTLV-II antigens [35]. With up to 60% similarity in identity, HTLV-3 and HTLV-4 has been incorrectly identified as either HTLV-I or HTLV-II serologically [36, 37]. Using the same HTLV blot 2.4 as this study, both negative and sero-indeterminate results were recorded before being confirmed as HTLV-3 [38]. Additionally, both HTLV-3 and HTLV 4 were discovered in Cameroun [39] which shares boundary, some cultural and geographical similarities with north-eastern Nigeria where this study was carried out. There is no existing literature on either HTLV-3 or HTLV-4 from north-eastern Nigeria. Both HTLV-3 and HTLV-4 have currently not been associated with any human disease [39] and it is very likely to find them among apparently healthy adults such as prospective blood donors.

More than half of the studies on HTLV carried out in Nigeria utilized only ELISA or other antibody detection methods as means of identifying the virus (Table 3) [40–49]. This has shown that many of the studies done in Nigeria are without confirmatory tests thereby leaving

Table 3: HTLV in Nigeria

Author Year Area	Method of Testing	Findings
Fleming et al. [16] 1986 Nationwide	Antibody detection (Not specified)	3.6% (North), 1.8% (Lagos) & 0.7% (Calabar) among blood donors
Williams et al. [14] 1993 Ibadan	ELISA and WB	11% among blood donors
Olaleye et al. [40] 1994 Nationwide	ELISA only	5.6% overall, 0.8% (children), 1.7% (adolescents), 16.3% (STD patients), 8.3% (Prostitutes), 6.4 (TB patients) and 3.3% (HCW)
Olaleye et al. [41] 1996 Ibadan	ELISA and PCR	5.4% overall, 15% (patients with STDs) & 1.5% (patients with leukaemia/Lymphoma)
Olaleye et al. [42] 1999 Ibadan	ELISA only	4.3% among mothers and 1.1% among their children

Table 3: *(Continued)*

Forbi JC and 2007 Ibadan Odetunde A [24]	ELISA only	5.1% (secondary school students), 16.1%, (pregnant women) and 22.9% (CSW)
Olusanya et al. [43] 1990 Ogun state	Antibody detection (Not specified)	0.0052% (healthy employees)
Terry et al. [15] 2011 Osogbo	ELISA and WB	3.6% among blood donors
Analo et al. [44] 1998 Lagos Okpara et al. [45] 1988 Cross-rivers	ELISA only Antibody detection (Not specified)	0.7% among blood donors 0.79% among blood donors
Durojaiye et al. [46] 2014 Lagos	ELISA and WB	1.% by ELISA and 0.5% by WB among blood donors
Okoye et al. [20] 2014 Enugu	ELISA only	0.5% among pregnant women
Akinbami et al. [47] 2014 Lagos	ELISA and WB	5.1% among patients with lymphoid malignancies
Oladipo et al. [48] 2015 Ogbomoso	ELISA only	25.8% among blood donors
Okoye et al. [19] 2015 Enugu	ELISA only	0% among blood donors
Opaleye et al. [49] 2015 Osogbo	ELISA only	24.2% among pregnant women

Abbreviations: ELISA Enzyme Linked Immunosorbent Assay

the possibility of having some false positive results among them.

CONCLUSION

The human T cell lymphotropic virus (HTLV) seroprevalence of 6.5% in this study which all turned out negative by western blot, further buttresses the need for confirmatory testing in HTLV research works. There is need to conduct a larger and community based research study on HTLV (including HTLV-3 and HTLV-4) in Gombe Nigeria. Comparative analysis of HTLV ELISA positive results with malaria testing will also help in revealing some possible explanations to the false positive ELISA in this malaria endemic zone. Routine screening of HTLV among prospective blood donors may possibly only be implemented based on regional realities from sound research works across the country because of size and geographical/socio-cultural variations in Nigeria.

SUPPLEMENTARY INFORMATION

Supplementary Information is available online at the journal's website.

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Author Contributions

Mohammed Mohammed Manga – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Adeola Fowotade, Yuguda Saleh – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Saleh Yuguda – Substantial contributions to conception and design, Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published

Girei Ahmed Iya – Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published

Mohammed Yahaya – Analysis and interpretation of data, Drafting the article, Final approval of the version to be published

Yakubu Munkaila Sheriff – Acquisition of data, Revising it critically for important intellectual content, Final approval of the version to be published
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Rasheed Ajani Bakare – Substantial contributions to conception and design, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor
The corresponding author is the guarantor of submission.

Conflict of Interest
Authors declare no conflict of interest.

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