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Prevalence of Human T-Lymphotropic Virus Infection Among Selected Blood Donor Facilities in Parts of Jigawa State, Nigeria

[1]Zainab Ahmad Adamu, Sani NM, [1]Bashir Sajo Mienda, [2]N.S Mujahid,[3]Abba Ahmed Danzomo, [4]Usman Muhammad Ibrahim

[1]Department of Microbiology and Biotechnology,
Federal University Dutse,
Jigawa State,
Nigeria.

[2]Department of Microbiology,
Aliko Dangote University of Science and Technology,
Wudil. Kano,
Nigeria.

[3]Global Health and Infectious Disease Control Institute,
Nassarawa State University,
Keffi,
Nigeria.

[4]Departments of Public Health and Community Medicine
Elrazi Medical University Kano,
Nigeria.

Abstract
Human T-Lymphotropic virus (HTLV-1) is a human retrovirus associated with several immune related disorders. This study aimed to determine the prevalence of human T-LV-1 among blood donors in Jigawa state, Nigeria. A descriptive cross-sectional design was used to study blood donors who presented at the blood bank of hospitals in three senatorial zones namely Dutse, Kazaure and Hadejia General Hospitals respectively selected using a systematic sampling technique. The data was analysed using SPSS Version 20 with statistical significance set at P≤5%. The prevalence of the virus in this study was 9(6%). Only 8 (5.3%) married respondents were positive. All the respondents that have undergone previous blood transfusion do not have the virus The burden of the virus among blood donors is significant and therefore a system of quality screening of all the blood donated to the blood bank should be enforced, an antigen based test will be better.

Keywords: Blood donors, Human-T-lymphotrophic virus, Prevalence, Serology, Jigawa, Nigeria

INTRODUCTION
The Human T-Lymphotropic virus (HTLV-1) is implicated in a number of diseases among humans. Epidemiological and molecular studies demonstrate a credible association between HTLV-1 infection and Acute T-Cell Leukaemia (ATL), which is a rare and aggressive T-cell

Author for Correspondence

cancer found commonly in areas where HTLV-1 is endemic, the risk of developing Adult T-cell Leukemia/lymphoma (ATLL) was greater with higher pro-viral load (the percentage of circulating CD4 T cells with integrated viral DNA) or higher anti-HTLV-1 antibody levels (Sertoz *et al.,* 2010).

Since 2011, HTLV-1 screening and confirmation is recommended to all pregnant women in Japan (Moriuchi *et al.,* 2013), and other countries as an important approach for the prevention of mother to child transmission of the disease. The pregnant mothers are advised to use either exclusive formula feeding, freeze–thawing of expressed breast milk, or breastfeeding for a maximum of 3 months and to test their offspring for HTLV-1 antibodies at three years of age, unless they give birth to high-risk infants such as premature babies, for whom breastfeeding is justified (Moriuchi *et al.,* 2013). Method to prevent milk-born infection and it will reduce incidence of Adult T-cell Leukemia/lymphoma (ATLL) patients among individuals born from HTLV-1 carrier mothers (Moriuchi *et al.,* 2013).

In 2011, (Alli *et al*., 2011) found 3.6% seroprevalence of HTLV among blood donors in South-Western Nigeria it was found 0.5% prevalence among blood donors in 2014 (Durojaiye *et al.,* 2014). Out of the four neighbouring countries of Nigeria, Cameroon seems to be the only country with relatively recent reports of HTLV incidence (Anyanwu *et al.,* 2018), Niger and Benin Republics have reports from 80s and 90s. The established modes of transmission of the virus include transfusion of an infected blood, sexually acquired, transmission from mother to her baby, organ donation, use of contaminated sharps among other modes similar to HIV infection (Okoye *et al.,* 2014). Sociodemographic factors that have been implicated in high prevalence of HTLV-1/2 infections include socioeconomic status, geographical location, gender (female), age (older), marital status, promiscuity, recurrent sexually transmitted diseases (STDs) (Okoye *et al.*, 2014).

It has been a number of years after first identifying human T-Lymphotropic virus, HTLV-1 infection, however, studies on the burden, distribution, risk factors were studied across the industrialized countries. There is a paucity of data on the prevalence of HTLV-1 in Jigawa State, and Nigeria at large. Furthermore, screening of blood and blood components among blood donors in Jigawa State for human T-Lymphotropic virus, before blood donation is not fully operational with emphasis on infections notably HIV, hepatitis B virus (HBV), Syphilis, hepatitis C virus (HCV). Despite the fact that Human T-Lymphotropic virus is a public health problem, therefore it is very important to identify the burden among blood donors in Jigawa state. This will provide a baseline information that could be used in controlling the infection. The information could also be used to create public awareness against the risks of contracting Human T-Lymphotrophic virus in Jigawa state Nigeria.

Materials and Method

Ethics

Ethical approval was obtained from the Health Research Ethics Committee of the Jigawa State Ministry of Health with approval number MOH/SEC/L.S/558/VI dated 17th April, 2021. All the principles of research ethics were adhered to during the conduct of the research.

Study area

The state is one of the north-western states of Nigeria. There are a number of primary, secondary and tertiary facilities across the 27 Local Government Areas (LGAs) of the state providing different type of healthcare services including laboratory and blood transfusion

services. There are over 7 million people in the state in 2024 based on the projected population growth and are predominantly Hausa and Fulani and mostly farmers. The state has availability of agrarian land.

Collection of Samples

A minimum sample size of 150 was determined using the Reed-Frost model formula as describe by Svensson *et al.,* 2006. Using n, as the minimum number of samples to be collected, Z = standard normal deviate at 95% confidence interval= 1.96, p = prevalence from previous study= (15.0%=0.15) (Mohammed *et al.,* 2018), q= complimentary probability = (1-p): (1-0.15) =0.85.

Up to 2.5mls of blood samples were collected from eligible participant using a sterile needle and syringe and transferred in to EDTA container. Centrifuge samples for 30 minutes at 3000xg to obtained plasma. Plasma was then stored at 2-8°C. Repeated freeze-thaw cycles were avoided and particulates were removed by centrifugation ensuring that all precautions were taken in order to obtain reliable result

Study design and population

The study utilized a cross- sectional descriptive design involving blood donors who presented at the blood bank of hospitals in three senatorial zones; Dutse, Kazaure and Hadejia General Hospitals respectively in Jigawa state from March, 2019 to April 2020 recruited for the study using a systematic sampling technique.

Sampling Technique

Eligible respondents from the selected facilities were equally allocated. A systematic sampling technique was used to study the eligible respondents daily until the equally allocated sample size in each of the facility was obtained.

Screening of Blood Donors

The processes involved in selection of suitable donor were based on description by described (Baker. 1980).

Medical history and personal details of the donor information was obtained, whether was a high risk donor or not. Only those who donated blood for the first or second time in that year, within the age of 18-65 years of either sex, weight greater than or equal to 45kg, and a normal blood pressure of ≥ 90/50mmHg.were considered eligible. The selected donors were strictly screened and ensured to be healthy, not anemic, while poorly hydrated or those having any evidence of malnutrition or pregnant women were usually not considered for donation of blood. Basic physical examination of the donor.

The temperature readings were ensured to be within the acceptable range, pulse rate of less than 100 beat per minute and must be regular, haemoglobin level for male donor of at least 13.5g/100ml (135g/l) and above while the considered level for female donors was at least 12.5g/100ml *and* above, or haematocrit of 39% and above for male, while 36% and above for female.

Laboratory Analysis

Procedure for Full Blood Count

The full blood count was carried out on the same day of sample collection. Full blood count was carried out using Sysmex KN-21N (manufactured by Sysmex corporation Kobe, Japan) similar to what was done by (Mohammed *et al*, 2016) using a three-part auto analyzer that was able to run 19 parameters per sample of patient like haemoglobin concentration and or packed cell volume (PCV), platelet parameters, red blood cell concentration, mean corpuscular hemoglobin, white blood cells, mean cell volume, and mean corpuscular hemoglobin concentration,. Well mixed blood sample was aspirated by letting the equipment sampling probe into the blood sample and then pressing the start button. Approximately 20ul of blood was aspirated by the auto analyser (Mohammed. 2016). The results of the analysis were displayed after about 30 seconds and the printout copy of result was released on the thermal printing paper (Mohammed., 2016).

Determination of HTLV-1 using ELISA Technique

About 50µl of negative and positive controls were added to the positive and negative control wells in line with the guideline provided by the manufacturer of the kits similar to what was done by (Yahaya et al, 2019), whereby in the sample wells, an amount of 40µl sample dilution buffer and another 10µl sample was added. Similarly, the samples were added to the bottom without allowing it to touch the wall of the well. It was then mixed very well with gentle shaking. After about 30 minutes of incubation at an average room temperature of between (20-22^{0}C), it was sealed with closure plate membrane, diluted with a buffer several times for 96T, and then washed up to 5 times as conducted by (Yahaya et al., 2019). About 50µl HRP-Conjugate reagents were added to each well with the exception of the blank control well, it was then incubated for up to 30 minutes and then washed thoroughly. About 50µl each of Chromogen Solution A and Solution B were added to each well, then mixed by gentle shaking and incubated at 37^{o}C for about 15 minutes, avoidance to the light exposure was ensured during colouring. Further, 50µl of stop solution was added to each well to end the reactions, and colour change in the well was observed. Absorbance was read at 450nm using a microtiter plate reader and OD value of the blank control well is set as zero (Yahaya *et al*.,2019).

Data Analysis

The data collected were entered into Microsoft Excel for Windows, cleaned and analysed using SPSS Version 20 for Windows. Quantitative variables were summarized using measures of central tendencies, dispersion, frequencies, and percentages as appropriate. The outcome variable was presence of HTLV-1 defined as either positive or negative. The Chi-squared test was used was used to find factors associated with HTLV-1 status, Analysis of Variance (ANOVA) was conducted between the haematological parameters (red blood cells, white blood cells, platelete among others) and the outcome variable with statistical significance set at P ≤5%.

RESULTS

Table 1 shows the prevalence of Human T LymphotrophicVirus, out of the 150 blood donors used in the study. Out of the total number of blood samples collected from the eligible respondents, though not statistically significant, only 9 (6.0%) were HTLV-1 reactive while 141 (94.0) of the blood donated were not HTLV reactive, with p-value > 0.623.

Table 1: Prevalence of Human T LymphotrophicVirus among blood donors in parts of Jigawa state

HTLV-1 Test	Frequency	Percentage frequency (%)	P-Value
Reactive	**9**	**6.0**	**0.623**
Non-reactive	141	94.0	
Total	150	100	

HTLV-1= Human T-lymphocyte virus.

Table 2 shows that out of the total respondents studied, up to 31 (20.7%) attended Islamiyya, 21 (14.0%) had Primary education, while 83 (55.3%), and 15 (10%) had secondary and tertiary education respectively. Also the Hausa tribe was the majority of the respondents and among the 9 people that had a positive result 8 of them were Hausa while one of them was a Yoruba. Furthermore majority of the respondents in this study were married and among the married 8 of them had human T-Lymphotropic virus. The p-value of the socio-demographic characteristics were greater than 0.05 which shows no statistically significant relationship between the socio-demographic factor and having infection with human T-Lymphotropic virus.

Table 2: Distribution of Human T-Lymphotrophic (HTLV-1) in relation to socio-demographic factors

Variable	Frequency (%) No. Examined	Frequency (%) Positive	Frequency (%) Negative	*P-value*
Education Status				
Islamiyya education	31 (20.7)	2 (1.3)	29 (19.3)	1.0
Primary	21 (14.0)	1 (0.7)	20 (13.3)	
Secondary	83 (55.3)	5 (3.3)	78 (52.0)	
Tertiary	15 (10)	1 (0.7)	14 (9.3)	
Tribe				
Hausa/Fulani	141 (94.6)	8 (5.4)	133 (95.0)	0.4
Yoruba	8 (5.4)	1 (0.7)	7 (4.7)	
Marital Status				
Married	135 (90)	8 (5.3)	127 (84.7)	0.623
Single	8 (5.3)	1 (0.7)	7 (4.7)	
Widow	7 (4.7)	0 (0.0)	7 (4.7)	
Age of respondents				
20-25	60 (40.0)	3 (2.0)	57 (38.0)	0.916
26-30	44 (29.3)	3 (2.0)	41 (27.3)	
31 and Above	46 (30.7)	3 (2.0)	43 (28.7)	
Occupation of the respondents				
Farming	16 (10.7)	1 (0.7)	15 (10.0)	0.959
Fishing	50 (33.3)	3 (2.0)	47 (31.3)	
Government Workers	30 (20.0)	1 (0.7)	29 (19.3)	
Others	54 (36.0)	4 (2.7)	50 (33.3)	

Table 3 shows that the risk factors for human T-Lymphotropic virus 64% of the respondents said they are not sexually active while 36 % said they are sexually active (P > 0.05), 6% of those who said they were not sexually active had the virus while 3% do not have the virus, also 8

(5.3%) of the respondents have had blood transfusion, none of the people who had blood transfusion had the virus. Few of the respondents 15 (10%) in the study had delivery complication and none of those who had delivery complications had the virus (p> 0.05). also few of the respondents 7(4.7%) had a history of organ transplant. Among those who had the transplant, none of them was found to have the virus (p >0.05), while, 8 (5.3%) of the respondents reported using drug intravenously out of which one of them had the virus (p < 0.05).

Table 3: Distribution of Human T-Lymphotropic virus in relation to the Risk factors among blood donors in Jigawa State.

Parameter	Not. Examined (%)	Positive (%)	Negative (%)	*P-value*
Sexual Activity				
Yes	54 (36.0)	3 (2.0)	51 (34.0)	1.0
No	96 (64.0)	6 (4.0)	90 (60.0)	
Blood Transfusion				
Yes	8 (5.3)	0 (0.0)	8 (53)	1.0
No	142 (94.7)	9 (6.0)	133 (88.7)	
Delivery Complications				
Yes	15 (10)	0 (0.0)	15 (10.0)	0.6
No	135 (90.0)	9 (6.0)	126 (84.0)	
History of Organ Transplant				
Yes	7 (4.7)	0 (0.0)	7 (4.7)	0.64
No	143 (95.3)	9 (6.0)	134 (89.3)	
Intravenous Drug Usage				
Yes	8 (5.3)	1 (0.7)	7 (4.7)	0.40
No	142 (94.7)	8 (5.3)	134 (89.3)	

Table 4 shows that the total sum of squares of PCV 4452.088 while the total Sum of Squares of Hb 4452.088, total sum of squares of WBC 3555.031, total Sum of squares of LYM 76679.397, total sum of squares of MCV 5426.232, Total Sum of squares of MCH 459.918, Sum of Squares of MCHC 555.402, total sum of squares of Platelet 3219638.000, Total Sum of Squares of RBC count 149.070, total sum of squares of MXD 3965.927, total sum of squares of Neutrophil 21963.987, and none of the value are significant (≥0.05).

Table 4: Analysis of Variance between Hematologic parameters and prevalence of HTLV-1

		Sum of Squares	Mean Square	F	Sig.
PCV	A	21.003	21.003	.702	.404
	B	4431.085	29.940		
Hb	A	1.033	1.033	.558	.456
	B	274.038	1.852		
WBC	A	11.580	11.580	.484	.488
	B	3543.450	23.942		
LYM	A	6.124	6.124	.012	.914
	B	76673.273	518.063		
MCV	A	153.123	153.123	4.298	.040*

	B	5273.108	35.629		
MCH	A	.356	.356	.115	.735
	B	459.561	3.105		
MCHC	A	9.017	9.017	2.442	.120
	B	546.385	3.692		
PLATELET	A	23702.700	23702.700	1.098	.296
	B	3195935.300	21594.157		
MXD	A	53.839	53.839	2.037	.156
	B	3912.087	26.433		
	Total	3965.927			
RBC	A	.639	.639	.637	.426
	B	148.431	1.003		
Neutrophil	A	10.316	10.316	.070	.792
	B	21953.671	148.336		

KEYs:*= Significant difference; **PCV**=Packed cell volume; **Hb** = Hemoglobin Concentration; **WBC**=White blood count; **LYM**= lymphocyte percentage; **MCV**= Mean Corpuscular Volume; **MCH** = Mean Corpuscular Hemoglobin; **MCHC =** Mean Corpuscular Concentration**; MXD = Mixed** cell percentage**; RBC=** Red blood cell**; Sig =** P-value

DISCUSSION

The study was conducted to find the prevalence of human T-Lymphotropic virus among blood donors in Jigawa state, Nigeria. The HTLV have shown gradual, but considerably consistent, increase in prevalence since their discovery (Anyanwu*et al.,* 2018). HTLV-1 subtypes were associated with specific regions of the globe, while HTLV-2 subtypes are related to highly specific subpopulations and behaviour like injection drug use (Anyanwu *et al.*, 2018). In Gabon, 4.2% of the population studied were found to have the virus (Ramassamy *et al*., 2020)

The results obtained in this study with respect to the risk factors and socio-demography is in accordance with that of (Manga *et al* .,2016) which reported that the major risk factors for HTLV-1 seropositivity among participants include; age less than 30 years, marital status and previous history of blood transfusion. Other significant risk factors include; first time commercial blood donors and drug addiction. Prevalence data are available from different African countries reported ranges from 0% to 1.2% among blood donor studies, 1.2% to 2.2% in studies of pregnant women (Armah *et al*., 2006) Also zero prevalence rates have also been reported in other parts of Africa like Mali and Benin Republic (Houinato*et .al.*, 2007). We also found 3 respondents (2%) who were sexually active, and 6 respondent (4%) who were not sexually active were both HTLV-1 positive thus suggesting that there is no correlation between sexual activity and contracting the virus.

The prevalence of HTLV-1 based on socio-demographic characteristics in this study is 9 (6%) and those married respondents that are positive were 8 (5.3%) which is similar to other studies (Ita *et al*., 2014). Transmission from men to women has been observed to occur more efficiently than from women to men in these longitudinal studies as well as in some cross-sectional studies that found male negativity to be more common than female negativity in sero-discordant couples.Longer duration of a couple's relationship and older age of a seropositive male partner have also been associated with a higher risk of transmission (Kinbami *et al*., 2014) Higher viral load was a risk factor for transmission in both cross-sectional and longitudinal studies. Another group of studies reported on couples or sexual partners involving HTLV-1-

seropositive individuals to determine the transmission risk and co-factors associated with transmission. In this study, all the respondents that have undergone blood transfusion do not have the virus, this has negate the popular conclusion that Transfusion of cellular blood products carries a very high risk of transmission (up to 60%), as does solid organ transplant which strongly suggest that proper blood screening before transfusion can significantly reduce the chances of transmission of the virus.

However, transfusion of cell-free plasma carries a low or no risk of transmission. The risk of nosocomial transmission or transmission associated with injection drug use is unknown. It is well established that higher HTLV-1 proviral load is a risk factor for transmission, but the risk associated with very low or undetectable levels remains unknown. Prevention strategies explicitly targeting HTLV-1 transmission remain limited to screening of blood donations. This study has also shown us that among those respondents with complication during delivery, none of them were infected with the virus, this is in line with several discovery that the totality of evidence suggested that mother-to-child HTLV-1 transmission occurs primarily through breastfeeding, with limited evidence of intrauterine transmission or transmission during delivery (Cárdenas-Roldán et al., 2013).

Hematological parameters, like complete blood count (CBC) results, can provide insights into the systemic effects of infection. Hematological abnormalities associated with HTLV-1 infection can include lymphocytosis (an elevated number of lymphocytes), monocytosis (an increased number of monocytes), and thrombocytopenia (a reduced number of platelets) .In terms of prevalence, HTLV-1 infection is more common in certain regions of the globe, particularly in like parts of Japan, South Africa, the Caribbean, Southern America, and sub-Saharan Africa. It was estimated that small proportion of infected individuals could go on to develop diseases, like leukaemia, lymphomas and other haematological malignancies (Ribeiro *et al*., 2022).

CONCLUSION

The prevalence of Human T-Lymphotropic virus among blood donors in the selected hospitals in Jigawa state was found to be 6%, exposing the importance of confirmatory assays after negative antibody detection assay results. The risk factors that have been suggested in the study are sexual activity, blood transfusion, delivery complication, history of organ transplant and intravenous drug usage, out of all the risk factors, the most important risk factors in this research were sexual activity and intravenous drug usage because some of those respondents that were involved were also infected with the virus. The majority of the respondents in this study were Hausa/Fulani tribe with secondary school education, also majority of them were married and their major occupation was farming and fishing although the presence of virus does not show any association with the occupation of the respondents.

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