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ORIGINAL PAPER

First detection of human T-lymphotropic virus in blood donors in Benin shows that testing is required to improve blood safety

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Introduction Human T-lymphotropic virus (HTLV) is a blood-borne infection that can be transmitted via blood transfusion. Knowing that blood safety can improve blood transfusion to prevent dissemination of viral infections in medical facilities, there is no routine pre-transfusion screening for HTLV in all blood banks in the Republic of Benin. This study aims to estimate the prevalence of HTLV infection in blood donors and describes the characteristics of positive donors.

Methods A HTLV prevalence study was carried out by screening 2,035 samples by an enzyme-linked immunoassay obtained from six blood banks located throughout the Republic of Benin. The PCR method was used to confirm and type all the ELISA reactive samples.

Results and discussion Twelve subjects, all volunteer blood donors, were found with positive serology confirmed by a specific HTLV type 1 PCR assay, representing an overall seroprevalence of 0·59%. Furthermore, seven subjects were indeterminate for anti-HTLV-1/2 antibody and only one sample was confirmed positive for HTLV type 1 in a PCR reaction. These are the first cases of HTLV detection among blood donors in Benin Republic, whose blood was already transfused to recipients. This result emphasizes that HTLV needs to be considered as a Public Health issue in the Republic of Benin.

Conclusion This study reports positive result of HTLV infection among blood donors in the blood banks of Benin Republic in West Africa and highlights the inclusion of screening tests and strategies to reduce its transmission.

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Key words: Blood donors, HTLV, HTLV prevalence, HTLV testing.

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Introduction

Although four types of human T-cell lymphotropic virus (HTLV) have been described, only HTLV type 1 has been associated with a wide spectrum of disease, including the lymphoproliferation of CD4-activated cells, known as

adult T-cell leukaemia/lymphoma (ATL), a chronic myelopathy called tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM), in addition to other inflammatory diseases [1–4]. Epidemiological data estimate that approximately 5–10 million people are infected with HTLV-1 worldwide, mainly concentrated in areas of high endemicity, such as Sub-Saharan Africa, Europe, Southeast Asia, Japan, the Caribbean, and in both the American continents [5].

Sub-Saharan Africa is considered to be one of the largest areas of endemicity for HTLV-1 infection, comprising an estimated 2–4 million infected individuals [6]. However, most of the early sero-epidemiological studies of HTLV-1/2 infection performed in Benin, a country situated in West Africa shown the prevalence of 1·5%, 1·86% and no positive individuals for antibody to HTLV-1, respectively, among the general population of Benin, the Department of Atacora in north-western Benin and the blood donors [7, 8]. Despite previous data indicating the presence of HTLV-1 in the general population of Benin, HTLV-1 antibody screening is not routinely done at the *Agence Nationale pour la Transfusion Sanguine* (ANTS) of Benin Republic. Moreover, knowing that HTLV and HIV share important homologous aspects of transmission including sexual contact, breastfeeding and blood transfusion, HTLV still remains a relatively hidden public health problem in some part of the African continent, largely due to the lack of the knowledge about this retrovirus.

Blood is an essential transporter for oxygen, nutrients and other substances throughout human tissues. Blood donation could be life-saving for individuals who have lost blood during accidents, surgery or due to severe low platelet count, and severe anaemia observed with infectious diseases such as malaria and HIV infection. Therefore, screening blood donors for blood-borne infections may help maximize safety of blood donation for the donor and the recipient [9]. While screening of blood donors is therefore crucial to prevent dissemination of the virus in medical facilities [10], few studies have been conducted on HTLV-1 related to blood safety, in order to indicate the risk of HTLV transmission by blood products and to improve blood safety in Benin.

The *Agence Nationale pour la Transfusion Sanguine* (ANTS) of Benin Republic currently selects unpaid, voluntary blood donors on the basis of a health check questionnaire and examination performed by a physician. All donated samples are then tested for blood-borne infections, such as human immunodeficiency virus (HIV), hepatitis B and C (HBV, HCV) and syphilis according to WHO recommendations at each branch of ANTS. Presently at the Benin Republic ANTS, there is no routine pre-transfusion screening for HTLV, knowing that HTLV is also a blood--borne infection transmissible in a similar manner to all others virus which are already tested in Benin Blood banks.

Given that the major challenge of the Ministry of Health in Benin is the provision of high-quality blood products that meet World Health Organization (WHO) standards to ensure blood safety, the present study was designed to estimate the prevalence of HTLV infection in blood donors and describes the characteristics of positive donors so as to fulfil the complex goals of Benin blood banks, such as providing blood products to reduce the mortality of endemic diseases such as severe malarial anaemia, in a safe and effective manner.

Methods

Study design and population

A cross-sectional descriptive study was conducted using samples from volunteer blood donors who donated blood between July and December 2015. The samples were collected at the six provincial branches of the *Agence Nationale pour la Transfusion Sanguine* (ANTS) of Benin Republic: Atacora/Donga (AD), Atlantique/Littoral (AL), Borgou/Alibori (BA), Mono/Couffo (MC), Ouémé/Plateau (OP) and Zou/Colline (ZC). The localization of the six provinces in Benin Republic are presented in the supporting Information. All included participants were volunteer blood donors older than 18 years old who donated blood during the study period.

This study received approval from the Faculty of Science and Health (Benin University), and informed written consent was obtained from each subject. The subjects were interviewed to obtain sociodemographic information.

Sample size and collection procedures

The sample size required was determined using the Schwartz formula:

$N_1 = \varepsilon^2 pq/i^2$, where N_1 represents the minimum sample size required; ε is the standard normal deviation at a 95% confidence interval corresponding to 1·96; p is the assumed true population prevalence of HTLV infections in Benin in 1989; q is the probability of not having HTLV; i is the absolute error between the estimated and true population prevalence, considering the following values: $P = 0{\cdot}015$ (HTLV prevalence in Benin in 1989), $q = 1-p = 0{\cdot}985$ and i = 1% (precision), $N_1 = 568$.

Serologic testing

The blood samples used in our study were collected in an EDTA tube at the time of the blood donation in each provincial branch of ANTS of Benin. The collected blood

samples were stored in an icepack and were transported on the same day to a laboratory for separating into plasma and peripheral blood leucocytes (PBLs). The plasma samples were initially screened by an enzyme-linked immunoassay (ELISA) to detect antibodies to HTLV-1/2 using a Murex HTLV 1/2 diagnostic assay at a specificity of 99·5% (Murex Diagnostics, DiaSorin, Dartford, UK) in accordance with the manufacturer's instructions. Initially reactive samples were confirmed by repeat testing using the same ELISA, and only repeat reactive samples were considered positive.

Polymerase chain reaction for confirmatory and typing analysis

Peripheral blood DNA was extracted from the buffy coat of the ELISA reactive sample using QIAamp DNA Blood kit, QIAGEN GmbH, Hilden, Germany, in accordance with the manufacturer's instructions. HTLV-nested polymerase chain reaction (PCR) targeting the pol region has been performed to confirm the presence of proviral DNA and to differentiate between HTLV-1 and HTLV-2 as a reference [11]. The amplified products were analysed by 1% agarose gel electrophoresis followed by ethidium bromide staining.

Data analysis

Data were collected and transferred to a spreadsheet using Microsoft Excel 2013 for analysis using Epi Info™ 7 statistical software. Fisher's exact test was used to compare the proportions of donors presenting risk factors associated with HTLV using a level of significance of $P \leq 0{\cdot}05$.

Results

A total number of 2035 blood donors equal to or older than 18 years were included in our study. The average donor age was 26 years (ranging from 18 to 65 years), with 75% of donors aged <34 years. Most study participants were male 1675 (82·3%) and were between 18 and 26 years of age.

Table 1 delineates the number of blood samples obtained from each geographic location, as well as ELISA results. The supporting Information showed the prevalence rates among 2035 blood donors in Benin according to each provinces. We identified a total of 12 HTLV reactive results, providing an overall prevalence of 0·59% (95% confidence interval (CI) 0·259%–0·919%), and seven other subjects were considered borderline (DO/CO 1 << 1·23). All the 12 HTLV reactive samples have been confirmed as positive by PCR reaction and have been typed as HTLV-1. Considering the gender, among the male subjects, 9 (75%) were found to be reactive to HTLV antigens and 3 (25%) females were reactive to HTLV. The high prevalence is concentrated among the blood donors aged from 18–26 years and 27–34 years (41·67%), and this prevalence decreases with age 16·67% (35–45 years). According to occupation, our results show that 5 of 861 students (0·58%) were positive compared to 3 of 579 non-medical staff (0·52%), and 9 of 1369 singles blood donors were positive.

Table 1 HTLV reactive among blood donors in six blood bank provinces of Benin

Regions	No.	ELISA & PCR + (%)	CI 95%	Borderline (%)
Atacora/Donga	179	3 (1·68)	(0·18% to 3·16%)	2 (1·12)
Mono/Couffo	263	2 (0·76)	(−0·28% to 1·80%)	0
Zou/Colline	400	3 (0·75)	(−0·09% to 1·59%)	1 (0·25)
Atlantique/ Littoral	440	2 (0·45)	(−0·17% to 1·07%)	4 (0·91)
Oueme/Plateau	272	1 (0·37)	(−0·35% to 1·07%)	0
Borgou/Alibori	481	1 (0·21)	(−0·20% to 0·60%)	0
Total	2035	12 (0·59)	(0·259% to 0·919%)	7 (0·34)

It is also important to highlight that at least one individual tested HTLV reactive at each provincial blood bank and regarding participant occupation, students presented the highest percentage of reactivity (41·67%) (Table 2).

Screening for blood-borne infectious, such as human immunodeficiency virus (HIV), hepatitis B and C (HBV, HCV) and syphilis, was previously performed according to WHO recommendations at each branch of the *Agence Nationale pour la Transfusion Sanguine* (ANTS). No blood donors with positive serology for the other viral markers evaluated (HBS, HCV, HIV, TPHA) presented reactivity in HTLV screening (Table 2).

No significant differences were observed when Fisher's exact test was used to compare the proportions of donors presenting risk factors considering history of transfusion, breastfeeding with duration longer than 6 months and partner number associated with HTLV reactive individuals (P = 1·00; 0·099; 0·70 respectively).

Finally, our findings revealed the alarming information that reactive HTLV blood donations had already been transfused to some recipients because of the lack of HTLV screening for blood donors in the same blood banks.

Discussion

Our study is the first cross-sectional study which explores the seroprevalence rates of HTLV and found reactive samples among blood donors. The study included a panel of a high number of male blood donors confirming the effective participation of men in blood donation schedules and the fact that potential blood donors are

Table 2 Study population and prevalence rate of HTLV reactive according to study variables

Variables	N°	ELISA & PCR + (%)	P value*	OR (95% CI)
Age				
18-26	1042	5 (41·67)	0·025	0·0048 (0·00059-0·009)
27-34	498	5 (41·67)	0·025	0·0100 (0·00125-0·018)
35-45	364	2 (16·67)	0·157	0·0054 (−0·0021-0·013)
>45	132	0	-	-
Partner number				
≤3	336	1	0·70	0·45 (0·05-3·54)
>3	1692	11		
Sexo				
Male	1675	9 (75)		
Female	360	3 (25)	0·45	0·63 (0·17-2·37)
Civil status				
Married	626	3 (25)		
Single	1369	9 (75)	0·76	0·72 (0·19-2·69)
Occupation				
Student	861	5 (41·67)	0·025	0·0058 (0·00072-0·010)
Non-medical staff	579	3 (25)	0·083	0·0051 (-0·0006-0·011)
Driver	79	2 (16·66)	0·158	0·0253 (-0·0100-0·060)
Artisans ou Business	349	1 (8·33)	0·318	0·0028 (-0·0027-0·008)
Medical staff and soldier	123	1 (8·33)	0·319	0·0081 (-0·0079-0·024)
Others	44	0	-	-
Others viral markers				
HBS+	110	0		
HCV+	19	0		
HIV+	22	0	-	-
TPHA+	8	0		

*Fisher exact test for comparisons among blood donors.

predominantly male [12]. This may be a consequence of the low number of female blood donors evaluated in our study. The prevalence of infection among men is high compared to women. This is not consistent with other previous studies indicating a higher infection rate among women, which increases with age [13, 14]. In our study the seroprevalence rate found among blood donors was low compared to the prevalence among the general population of Benin, which was 1·5% in the six provinces of the country and 1,86% was reported in the Department of Atacora in north-western Benin [7, 8]. Our study is the first to report reactive sample in ELISA and confirmatory test using PCR methods among blood donors in Benin, since a national survey conducted in Benin between 1988 and 1989 (including over 1,300 donations) indicated no positive blood donors for the HTLV-1 antibody when the blood donors were screened by immunofluorescence and enzyme immunoassay and reactive samples were further confirmed by Western blot and RIPA [7]. Therefore, comparison with other studies among blood donors in Benin country would be difficult. The difference in the seroprevalence rate observed in this study and the study among the general population of Benin can be due to the number of blood samples very close or equal tested in our study compared to the previous study. Otherwise, it may be due to some limitations of the methods employed in our study and the lack of confirmatory tests (because of the difficulty to recruit presumed positive blood donors and have access to the samples for confirmatory tests).

Our study showed a heterogeneous presence of blood donors reactive to the ELISA test in all provinces of Benin, which was also the case with the study conducted in the general population of Benim [7]. These observations highlighted the importance of adding HTLV-1/2 screening in Benin blood banks to prevent the transfusion of contaminated blood with HTLV infection to recipients. The seroprevalence found in our study is relatively high in comparison to a study conducted among blood donors in nearby West African countries like Senegal, with a prevalence of 0·16%, but low in comparison to studies carried out in Nigeria and Mali that reported a seroprevalence of 3·2% and 1·4% respectively [6, 15, 16].

These preliminary data show that there are individuals considered as HTLV reactive for anti-HTLV-1/2 antibody

assessed using ELISA in Benin. Considering the current process of blood-borne infection screening applied in Benin doesn't include HTLV screening, our results confirm the need to include HTLV screening procedures for blood transfusion safety, similarly to what currently exists for HIV in Africa. In low-resource countries, such as Benin, in the absence of confirmatory test screening procedures, we propose that ELISA may be used, enabling any reactive samples to be exempted from availability for blood transfusion.

Blood-borne pathogens, such as bacteria and viruses, are present in blood and body fluids and can cause disease in humans. Safety of the blood supply from pathogens involves a multifaceted approach including screened blood units for transfusion transmissible infections. The surveillance of blood safety can reduce unnecessary transfusions, prevent transmission of blood-borne pathogens like HTLV through unsafe blood and make safe blood available in management of complicated pregnancies, severe anaemia, malaria, AIDS patients on ART, accidents and trauma, cancer treatment and haematological conditions. Furthermore, surveillance of blood transfusion for blood safety can reduce morbidity and mortality, following standard precautions to help prevent the spread of blood-borne pathogens and other diseases. These precautions require that all blood and other body fluids be treated as if they are infectious.

Finally, our findings revealed the alarming information that reactive HTLV bloods donations had already been transfused to some recipients because of the lack of HTLV screening for blood donors in the same blood banks. Screening of these recipients is in progress for serological and molecular confirmatory testing to verify the risk of seroconversion after receiving infected HTLV blood. We believe that the findings presented herein should demonstrate to public health policymakers of Benin Republic that it is important to improve blood safety by adding HTLV screening for blood donation in Benin in order to monitor the dissemination of the infection for public health surveillance. Further studies are needed to determine the type of HTLV present among the reactive blood donors and conduct follow-ups on the positively identified blood donors to monitor the progression of infection.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

A.E.R.A., M.C.R., S.K., L.A. and L.C.J.A. contributed to conception and design. A.B., D.D., D.D. and L.A. collected the samples and data. A.E.R.A., D.D., and A.B. performed the experiments. A.E.R.A., M.G. and L.C.J.A analysed the data. A.E.R.A., E.S., F.K.B., J.L., M.C.R. and L.C.J.A wrote the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. HTLV-1/2 serologic enzyme-linked immunoassay (ELISA) screening prevalence rates among 2035 blood donors in the Benin provinces.