ORIGINAL ARTICLE

Human T-cell lymphotropic virus type I and II seroprevalence among volunteer blood donors in Thailand

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ABSTRACT

Human T-cell lymphotropic virus type I and II (HTLV-I/II) are closely related but distinct retroviruses that can infect humans. Both the viruses can be transmitted via transfusion of contaminated blood components. HTLV pre-transfusion screening is not mandatory in Thailand until now. Current epidemiological data for HTLV prevalence is still lacking since the past surveys were done more than a decade ago. The main objective of this study was to determine the seroprevalence of HTLV-I/II among voluntary blood donors in Thailand. 11,057 volunteer blood donors were screened for HTLV-I/II antibodies using the ARCHITECT rHTLV-I/II chemiluminescent immunoassay (CLIA). Initial-reactive (IR) samples were subjected to repeat duplicate testing and were also sent for confirmatory testing at Korean Red Cross Society (KRC), Seoul or National Serology Reference Laboratories (NRL), Australia using alternate HTLV serological assays and immunoblot and/or specific nucleic acid testing respectively.

Out of 11,057 plasma samples, 10,080 were low-risk seronegative donors and 977 were first-time/high-risk donors. Twenty of 24 IR samples were repeatedly reactive (RR) in low-risk seronegative donors group. On confirmatory testing of these 24 IR by immunoblot, 13 indeterminate and 11 negative results were observed. One out of 977 samples from first-time/high-risk donors was RR for anti-HTLV-I/II antibodies. This sample was co-reactive for HBsAg, but negative for HTLV by EIA or in-house HTLV-I qPCR. The ARCHITECT rHTLV-I/II assay exhibited a specificity of 99.93% in low-risk donors and 99.90% among high-risk donors.

This study concluded that HTLV-I/II prevalence is low among blood donors in Thailand. But periodic surveillance should be continually conducted to ensure high blood safety standards in the country.

KEYWORDS

HTLV; seroprevalence; blood donors; Thailand; South-east Asia; retrovirus; blood screening; transfusion transmitted infection

Introduction

First discovered in the early 1980s [1], HTLV-I is known as the etiological agent of adult T-cell leukemia/lymphoma (ATL) and tropical spastic paraparesis (TSP), however, HTLV-II is not clearly associated with a known clinical disease [2,3]. Both HTLV-I and HTLV-II can be transmitted via infected cellular blood products transfusion, sharing of needles by intravenous drug users, and unprotected sexual intercourse [4,5]. Thus, in efforts to disrupt transfusion related transmission, antibody screening for HTLVs was introduced in many countries globally and remains in place till today. Many countries have implemented leucodepletion which can also significantly reduce HTLV infectivity because infected cells are leucocytes and free viral particles are rarely produced.

Almost 10–20 million people in the world are thought to be infected by HTLV-I/II. In Asia to date, incidence of some cases of HTLV-I/II have been reported across many

countries including China, Malaysia, Philippines, Indonesia and Vietnam, yet only two Asian countries, Japan and South Korea have implemented mandatory screening of blood donors for HTLV-I/II antibodies [6–11]. In many endemic regions, HTLV-I co-infections with other TTI pathogens have also been reported among the high-risk populations [10,12]. Existing data suggest low endemicity of HTLV-I in Thailand and Southeast Asia. There was a study showing no HTLV-I prevalence among northeast Thai population in 1997 [13]. While HTLV-1 seropositivity (3 HTLV-I seropositive out of 86 female prostitutes) was reported in Thailand in a study conducted in 1992 among women working in hi-end massage parlors that were frequented by foreign tourists, which may suggest that HTLV-1 prevalence may vary among population subtypes [14].

In Thailand, the national strategies to ensure the provision of safe, high quality blood and blood products for all patients requiring transfusion form the

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basis of a nationally coordinated Blood Transfusion Service. Over the last few years, there has been substantial increase in the population migration and tourism across countries in Asia. Hence it is important for the National Blood Transfusion Services to evaluate threats of emerging and re-emerging diseases that can be transmitted through blood transfusion to ensure safe blood supply to the patients. Since HTLV blood donor screening is currently not being performed routinely in Thailand and the last reports regarding HTLV sero-epidemiology were published more than a decade ago [13–15], this study was undertaken to re-assess the sero-prevalence of HTLV I/II among blood donors in Thailand.

Methods

Study design and population

A total of 10,080 residual plasma samples from routine screening of voluntary blood donations which were screened negative for HIV, HBV, HCV and syphilis serology and MP NAT (pool of 6) markers from the National Blood Centre (NBC) in Bangkok, and 4 Regional blood centers (RBC) located in Chiang Mai (North), Nakornratchasima (Northeast), Lopburi (Central) and Phuket (South), were randomly selected for 3 months' period and included in this study and were categorized as low risk donors. Further, a total of 977 stored samples from last one year at NBC which screened positive for either HIV, HBV or Syphilis serology and NAT markers from first time donors (FTD) were also included in this study as high risk donors. Lipemic and hemolyzed samples were excluded from the study. All donors signed a donor application form authorizing storage and usage of their residual plasma samples in downstream research. The study protocol was approved by the Thai Red Cross Society (TRCS) Ethical Review Committee and the study was carried out at National Blood Centre in Bangkok from April 2013 to October 2014.

Serologic testing

Figure A1 illustrates the algorithm used for testing undiluted fresh (for low risk group) and frozen (for high risk group) residual plasma samples for HTLV-I/II antibodies using the Abbott ARCHITECT rHTLV-I/II chemiluminescent assay (CLIA; Abbott Laboratories, IL). Frozen samples were thawed and prepared for testing according to manufacturer's guidelines. Initially reactive (IR) samples were retested in duplicate. IR samples in low risk donor group were further tested on the Abbott PRISM HTLV I/II assay (CLIA; Abbott Laboratories, IL) and Immunoblot (MP Biomedicals, Singapore) at the Korean Red Cross Society (KRC), Seoul. And repeatedly reactive (RR) samples in high

risk donor group were further subjected to HTLV testing on Murex HTLV I + II enzyme immunoassay (EIA; DiaSorin Ireland) and an in-house quantitative HTLV-I real time polymerase chain reaction (HTLV-1 qPCR) (for research-use only) [16,17] using whole blood at the National Serology Reference Laboratory (NRL) Australia. For the HTLV viral load real time PCR, DNA is extracted from whole blood and amplified using HTLV-I specific primers and albumin as an internal control. The lower limit of detection of the PCR assay is 325 copies/mL of blood.

Results

The demographics characteristics of the study participants were summarized in Table A1. More than half of the study participants were males in both the low risk (51.4%) and high risk (63.36%) donor groups. The majority of donor age distribution ranged from 21 to 60 years with the highest proportion being in the 31–40-year range in both the study groups.

Of the 10,080 samples tested from low-risk donors, 24 samples were initially reactive but only 20 (20 out of 24 IR) samples were found repeatedly reactive, giving a specificity of 99.93% in low-risk donor samples (Table A2). All 24 IR samples were tested for confirmation with immunoblot and Prism HTLV I/II. 13 out of 24 IR (54%) were indeterminate and rest 11 (46%) were negative on immunoblot and all 24 IR samples were non-reactive on Prism HTLV I/II assay.

An additional 977 samples from first-time, high-risk donors seroreactive for TTIs (300 HIV positive, 460 HBV positive and 217 Syphilis positive) were tested. From this group of donors, only one sample was IR and RR reactive for anti-HTLV-I/II antibodies. This donor sample was previously confirmed reactive for HBsAg, and was tested for HTLV-I/II antibodies by NRL Australia using EIA and in-house HTLV-I qPCR, both tests yielded negative results (Table A3). The ARCHITECT assay thus showed a specificity of 99.90% among high-risk donors (Seroreactive for HIV, HBV, or Syphilis).

Discussion

Since the introduction of anti-HIV screening in 1986, the TRCS has helped to establish national strategies to keep the blood supply safe from transfusion transmitted infections in the country. Blood screening practices across Asia Pacific countries commonly include testing for HBV, HCV, HIV and Syphilis with only South Korea and Japan testing for HTLV additionally at the national level. HTLV is transmitted vertically through breast milk, or horizontally by sexual transmission or percutaneous exposure to blood and blood products. Historical Sero-epidemiological surveys from 1989 to 2000 among blood donors and various patient groups have failed to detect any HTLV prevalence in Thailand [13–15].

Recently, HTLV-I infection was reported in a Thai patient with chronic progressive myelopathy with the history of blood transfusion [18]. Given the growth in tourism and labor migration between Thailand and HTLV-endemic countries since these last studies reported, this present serological survey was conducted to assess the prevalence rate of asymptomatic disease or carriage status among voluntary blood donors.

Blood donations from first-time and repeat donors were collected from every region of country by TRCS regional blood centers and screened for anti-HTLV antibodies. The cross-sectional sampling was evenly split between males and females. First-time donors and repeat donors in low-risk (seronegative for TTIs) and high-risk groups (seropositive for any TTIs) were sampled across the age range of 21–60 years. Sampling was highest in the age 31–40 year old, who would have been 17–26 years old at the time of the last reported serological survey [13]. The significance of this observation is that this age group would include those at or becoming of age for high-risk horizontal acquisition of HTLV after the last survey.

Of 10,080 low-risk donor samples tested, 24 were initially-reactive, and out of which 20 were repeatedly reactive. Confirmatory testing of all 24 reactive samples was performed by Korea Red Cross Society, who have implemented an HTLV screening program for their blood donors since 2009. The KRCS tested samples with the FDA-approved PRISM HTLV-I/II assay and confirmed with (a now FDA-approved) immunoblot assay, reported 13/24 (54%) indeterminate results and 11/24 (46%) negative. The reasons for indeterminate blotting patterns remain unclear. Several possible explanations have been proposed for the occurrence of HTLV-I/II seroindeterminates that include cross-reactivity to other known retroviruses or a novel virus, antibody responses to a malaria parasite with epitope homology to HTLV-I, a defective HTLV-I or HTLV-II, and low copy numbers of prototypic HTLV-I in the affected patient yielding the indeterminate antibody response [19]. The samples with indeterminate blot results were not further tested for HTLV-I pro-viral DNA which could have helped to identify true reactivity.

Screening of 977 high-risk donors detected only one repeat reactive HTLV-I/II sample from an HBV-infected donor. This repeat reactive sample was tested by NRL Australia using serology and an in-house qPCR test for HTLV-I proviral DNA and reported negative. While the in-house PCR assay is only specific for HTLV-I and could have missed HTLV-II in the sample, the confirmatory EIA for HTLV-I/II is part of an effective testing program in Australia where HTLV-I virus is endemic in indigenous Australians residing in Central Australia, as well as in nearby Papua New Guinea.

While the ARCHITECT assay demonstrated 99.93% specificity among low-risk Thai donors, cross-reactive antibodies to other viruses or pathogens were not investigated.

Earlier studies among Indonesians and Filipinos populations indicated a possible source of biological false reactive results from antibody cross reactivity between homologous epitopes on HTLV-I p19 and the *Plasmodium falciparum* Exp-1 protein [20–22]. The performance of this current third-generation ARCHITECT assay, reformulated in 2008 to replace viral lysate with recombinant HTLV gp21 proteins and gp46 peptides, was comparable to previous high-specificity reports [23]. This current study did not capture whether the sero-reactive donors were from regions endemic for *Plasmodium* species.

The results of this serological survey reaffirm that HTLV is not endemic in the healthy Thai donor population. The smaller sampling of high-risk donors was biased towards donors with sexually-transmitted infections (STIs), since the TRCS donor pre-screening processes exclude intravenous drug users (IDUs). A cross-sectional analysis among other non-donor key populations could thus confirm if HTLV is not a public health concern in Thailand, since studies in endemic countries report higher prevalence among prisoners and IDUs [10,12].

While the present data shows no evidence of established HTLV-I/II infection among Thai blood donors, regular population migrations of Thai laborers and other key populations to and from Asian and Middle Eastern countries endemic for HTLV-I/II suggest periodic surveillance will be useful to detect new emerging and re-emerging diseases and to consider implementing technologies like leucodepletion to protect the safety of the blood supply in Thailand.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix A

Table A1. Age and sex demographic characteristics for the study participants.

Sex	% Distribution	
	Low-risk donors	High-risk donors
Male	51.40%	63.36%
Female	48.60%	36.64%
Age (years)		
<20	6.01%	6.86%
21–30	26.42%	43.91%
31–40	29.34%	29.68%
41–50	25.25%	15.15%
51–60	12.65%	4.40%
>61	0.33%	0.00%

Table A2. Distribution of low-risk donors and HTLV-I/II results.

Sample Collection Site	Donor Sample (N)	ARCHITECT HTLV-I/II (IR)	ARCHITECT HTLV-I/II (RR)	PRISM HTLV-I/II (Reactive)	Immunoblot (Indeterminate)
NBC Bangkok	2,000	6	6	0	2
RBC Chiangmai	2,580	6	3	0	4
RBC Lopburi	2,000	5	4	0	5
RBC Nakornratchasima	2,500	7	7	0	2
RBC Phuket	1,000	0	0	0	0
Total	10,080	24	20	0	13

RBC = Regional Blood Centre.

Table A3. Distribution of high-risk donors and HTLV-I/II results.

TTI reactive	Sample (N)	ARCHITECT HTLV-I/II (IR)	ARCHITECT HTLV-I/II (RR)	DiaSorin Murex HTLV-I/II (Reactive)	HTLV-1 Real time PCR (In- House)
HIV	300	0	0	ND	ND
HBV	460	1	1	0	Not Detected
Syphilis	217	0	0	ND	ND
Total	977	1	1	Negative	Negative

ND = Not Done.

Appendix B

Figure A1. HTLV - I/II testing algorithm followed in the study.

*IR/RR of low risk group were tested for confirmation on PRISM HTLV and MP immunoblot and IR/RR of high risk group were tested for confirmation on Diasorin HTLV and In-house qPCR.