

HTLV-1/2 prevalence in two Amazonian communities

Elida CG Mata¹, Roberto M Bezerra², Aldo A Proietti Júnior², Luana KS Pamplona³, Lilian O Gomes⁴, Valmir C Corrêa⁴, Jordan SR Caluff⁴, Geanny S Borges⁴, J Casseb¹ and LIB Kanzaki^{1*}

¹ Laboratory of Bioprospection, Department of Pharmacy, Faculty of Health Sciences, University of Brasília, DF, Brazil

² Laboratory of Bioprospection and Atomic Absorption, Federal University of Amapá, Macapá, AP, Brazil

³ Nutritional Management Consulting, Belém, Pará, Brazil

⁴ Secretary of Health, Oiapoque, Amapá, Brazil

Abstract

Introduction: The human T-cell lymphotropic virus type 1 (HTLV-1) is aetiologically linked to myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukaemia (ATL) besides other less incident pathologies, while the type 2 has not been definitively linked to any diseases.

Objectives: To determine the HTLV-1/2 seroprevalence in two Brazilian communities in northern Brazil.

Methods: In 2010 and 2015, HTLV-1/2 serological surveys were carried out in the Oiapoque county at the Brazilian border with French Guiana and in Santa Cruz do Arari, Marajó Island. Serum and breast-milk samples from 317 women (pregnant, lactating and non-pregnant non-lactating) resident in the Oiapoque county, together with serum samples from 217 females and 70 males living in Santa Cruz do Arari county, were twice screened by two distinct commercial immunoassay methods for antibodies to HTLV-1/2. Seroreactivity was confirmed by a commercial Western blot technique. Participants were interviewed for data concerning their health, socioeconomic and educational status.

Results: None of the Oiapoque women, mostly young and descendants of migrants, had antibodies to HTLV-1/2, despite the high HTLV-1 prevalence in neighbouring French Guiana and Caribbean Islands, while five females and three males living in Santa Cruz do Arari county were HTLV-1 infected as confirmed by Western blot testing. In contrast, the Santa Cruz do Arari community lives in relative isolation and is descended mostly from black African people with high consanguinity.

Conclusion: Despite the proximity between Oiapoque and Santa Cruz do Arari counties, ethnic, age differences, community isolation and consanguinity may explain the distinct HTLV-1/2 epidemiology in these areas of northern Brazil.

Keywords: HTLV-1/2, pregnant/lactating women, Oiapoque, Marajó Island, Santa Cruz do Arari county, Northern Brazil

Introduction

The deltaretrovirus genus comprises agents infecting mammals including humans, simians and bovines [1]. Virions are mainly transmitted by sexual intercourse, via breastfeeding from mothers to infants and by blood transfusion. The human T-cell lymphotropic virus, HTLV, now grouped into four types (HTLV-1–4), presents distinct pathological features. The detection of HTLV-1 infection has been well documented in various human diseases and is thought to be aetiologically involved in neuromyelopathy initially described in 1985 by Gessain *et al.* [2] and named tropical spastic paraparesis (TSP) by Zaninovic *et al.* [3] in South America and HTLV-1-associated myelopathy (HAM) by Osame *et al.* [4] in Japan, and adult T cell leukaemia/lymphoma, first described in Japan by Takatsuki *et al.* [5].

Both HTLV-1 and HTLV-2 were initially isolated in the USA in Robert Gallo's laboratory by Poesz *et al.* in 1980, and Kalyanaraman *et al.* in 1982, respectively [6,7]. HTLV-1 infection occurs worldwide while HTLV-2 was initially mainly restricted to Amerindians and people who inject drugs on the American continent [8,9]. In Brazil, HTLV-1 and HTLV-2 prevalence changes according to geographical location, and is mainly determined by ethnicity. HTLV-1 prevalence is high in areas that have received waves of African people brought to Brazil as slaves, particularly Maranhão, Bahia and Pará states, with a prevalence in blood donors of 1.0%, 0.94% and 0.91%, respectively. The lowest HTLV-1 prevalence was found in Santa Catarina, 0.04%, predominantly populated by people of European origin [10,11].

There is a proportional correlation between populations of African and Japanese descendants and areas of high HTLV-1 prevalence in Brazil. Even in areas of low HTLV-1 endemicity, there is a

significant prevalence of HTLV-1 among Japanese immigrants, for example in central Brazil, Campo Grande, Mato Grosso do Sul state [12], or in areas of high endemicity such as northern Brazil Tomé-Açú, Pará state [13].

Few studies have been published regarding HTLV-1/2 prevalence in Amapá state in northern Brazil, near the border with French Guiana. Among Waiãpi Amerindians inhabiting the northwestern region of the Amapá state, we have found one in 94 individuals to be HTLV-1 infected as confirmed by immunofluorescence and Western blotting (WB) techniques, while members of other Amerindian ethnicities (Galibis, Kubenkroke, Mundurucus, Palikur and Yanomami) were negative for HTLV-1 antibodies [14].

In 2004, Pouliquen *et al.* [15] reported an HTLV-1 prevalence of 3.1% among blood donors in Guiana, and in 1999, Talarmin *et al.* [16], found a 0.59% HTLV-1 seropositivity among Amerindians in French Guiana.

In this study, we have determined the HTLV-1 seroprevalence in two municipalities in northern Brazil with an emphasis on women of reproductive age who are mothers, pregnant and/or breastfeeding.

Methods

Ethical approval

All participants who took part in the study gave authorization for blood and breast-milk testing, after signing the free and clarified consent terms included in the approval documents of the Committee of Ethics (registration number 175/09 and 042/02).

Amazonian communities

Two municipalities, Oiapoque (03° 51' 19.75" N/ 051° 47' 48.72" W) and Santa Cruz do Arari (00° 36' 00" S/ 49° 11' 00" W), located in the two neighbouring Brazilian states of Amapá and

*Corresponding author: LIB Kanzaki, SHIN QI 08, CJ 02, Casa 24, Lago Norte, Brasília, DF, CEP 71.520-220, Brazil
Email: kanzaki@unb.br

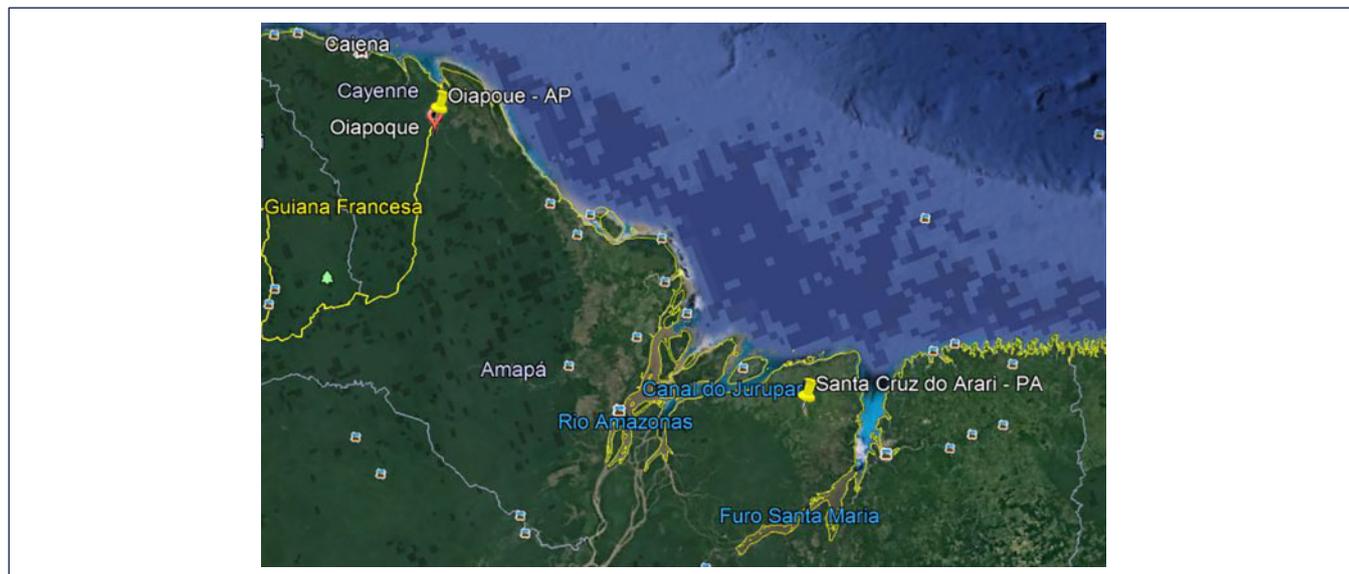


Figure 1. Map location of Oiapoque municipality in Amapá state, on the Brazilian border with French Guiana and Santa Cruz do Arari municipality in Marajó Island, Pará state.

Table 1. Age distribution of pregnant and breastfeeding mothers of Oiapoque county enrolled in the HTLV-1/2 epidemiological study.

Age	Reproductive status			Total
	Pregnant	Breastfeeding	Non pregnant/non breastfeeding	
0–15	12	4	1	17
16–20	61	13	1	75
21–30	100	31	7	138
31–40	40	20	5	65
41–50	3	4	3	10
>51	0	0	3	3
Total	216	72	20	308

Table 2. Age distribution and HTLV-1/2 positivity of Santa Cruz do Arari county population enrolled in the epidemiological survey.

Age	Female	Male	HTLV-1 positive	
			Female	Male
0–15	32	13	0	0
16–20	27	4	0	0
21–30	50	14	0	1
31–40	46	11	2	0
41–50	33	14	1	0
>51	29	14	2	2
Total	217	70	5	3

Pará, respectively, were selected for the serological survey (Figure 1).

The last population census in Amapá state dating from 2010, reported 669,526 inhabitants with Oiapoque county accounting for 3.06% (20,509 inhabitants) of the state population (<https://cidades.ibge.gov.br/v4/brasil/ap/oiapoque/panorama>). Santa Cruz do Arari county, located in the centre of Marajó Island, in the Pará state, has an urban population of 3994 inhabitants, representing 0.05% of the state population.

Population survey

The sero-epidemiological survey enrolled 317 women, representing 10.28% of women of reproductive age (15–39 years old) in the Oiapoque urban area (Table 1), and 217 females and 70 males in Santa Cruz do Arari county, representing 7.2 % of its population (Table 2).

All enrolled participants were interviewed and information regarding their educational, economic and health status was recorded. These data were used to produce a descriptive profile of the tested population.

Serological assays

Samples of serum and breast milk were tested twice for antibodies by two different ELISA kits: the Enzyme Linked Immunosorbent Assay (Abbot Murex HTLV I + II, UK), and the Gold ELISA

HTLV-I/II REM (Indústria e Comércio Ltda, SP, Brazil). Seroreactive samples were confirmed by a commercial Western blot assay, the HTLV-1/2 Blot 2.4 (Genelabs Diagnostics, Singapore).

Statistics

Descriptive statistics used data including the number of participants enrolled in the study, gender, socioeconomic and health status and the number of seropositive participants.

Results

None of the women from the Oiapoque region showed reactivity for HTLV-1 or -2 by immunoassay, using either serum or breast-milk samples, while five females and three males from the Santa Cruz do Arari county were reactive by ELISA testing and confirmed by WB (Table 2).

Of all the Oiapoque women participants enrolled into the study, 91.48% declared that they had lived in the Oiapoque region for the last 5 years, 5.7% in the Pará state, with the remaining from Goiás (Central-west region of Brazil), Maranhão and Paraíba states (Brazil northeastern region). Only 1.0 % had lived in French Guiana.

When considering their parents' origin, 41.9% were born in Amapá state, 34.4 % in Pará state (neighbouring state, Brazil northern region), 17.4% in Maranhão state (Brazil northeastern region) and the remaining in the states of Piauí, Ceará, Paraíba and Bahia (northeastern region of Brazil), Minas Gerais and Rio de Janeiro

Table 3. HTLV-1 prevalence among pregnant women in Brazil's main cities.

HTLV-1/2 Brazilian state	Number of pregnant women	Number who were HTLV-1/2 positive (%)	Assays used	Reference
Pará	13,382	0.29	ELISA, WB	26
Mato Grosso do Sul	116,689	0.11	ELISA, WB, PCR	27
Paraná	643	0.31	CMIA, PCR	28
Bahia	2766	1.05	ELISA, WB, PCR	29
Amazonas	674	0	ELISA, PCR	30
Alagoas	54,798	0.21	ELISA, WB	31
Ceará	814	0.12	ELISA, WB	22
Maranhão	2044	0.20	ELISA, PCR	32
Paraíba	1033	0.68	ELISA	23
Rio de Janeiro	1204	0.58	CMIA, WB	33
Espírito Santo	161	0.62	ELISA	34
São Paulo	913	0.11	ELISA, WB	35

PW: pregnant women; ELISA: enzyme-linked immunosorbent assay; WB: Western blot; CMIA: chemiluminescent immunoassay; PCR: polymerase chain reaction.

(Brazil southeastern region), and Amazonas (Brazil northern region). Some were born abroad in French Guiana (1%). In terms of ancestry, 14.5% were white European, 5.0% black African and 80.4% were of mixed black African/European heritage. Formal schooling data showed that 43.5% had finished or were enrolled into basic education, 43.8% in median education, and 11.0% in higher education. A minority (1.6%) declared themselves to be illiterate. Regarding professional or other types of activity, 68.1% declared themselves to be unemployed homeworkers, 7.3% unemployed students, 6.0% healthcare workers and the rest worked as janitors or government employees. When asked about their health, 75.4% reported no illness and the remaining participants declared themselves not to be in good health.

Among the participants from Santa Cruz do Arari, 94.5% stated that they had been living in the town for the last 5 years, while 3.5% had lived in Belém (Pará state), and the remaining in different communities in the Marajó Island. In terms of ancestry, 68.6% of the surveyed community were of mixed black African/European heritage, 21.4% were black African and the remaining 10.0% were European. When considering their parents' origin, 79.3% were born in Santa Cruz do Arari county, 4.1% in the neighbouring county of Cachoeira do Arari, 3.8% in Soure, both in Marajó Island, and 2.8% in Belém. The remaining parents were born in different communities in Marajó Island, apart from 1.4% who were from the neighbouring Maranhão state. Of the enrolled participants, 64.3% had finished basic education or were studying, 27.6% had completed or were enrolled in median education and 4.5% were enrolled or had finished higher education; 4.1% were illiterate. In addition, 28.8% were homeworkers, 24.5% students, 18.6% involved in fishing or farm labour and the rest were government employees, janitors or healthcare workers. More than half of the enrolled participants (52.1%) declared themselves in good health while the others reported poor health.

Discussion

The Oiapoque population in the urban area is composed of migrant people from the neighbouring Pará state and the Brazilian northeastern region, mainly attracted by gold mining and the vicinity of French Guiana, an overseas department of France (Figure 1). Despite high HTLV-1 prevalence in the Pará state, initially determined by ourselves [17–19] and other groups [20],

and in the northeastern area of the country [21–26], few participants from the Oiapoque county were of solely black African origin (5.0%) while most participants were of mixed European/black African heritage (80.4%). Our study sample size was small compared to other studies (see Table 3), representing 10.28% of women of reproductive age in the urban area. Other studies looking at HTLV-1 seropositivity among pregnant women (Table 3) have reported 39 HTLV-1 carriers among 13,382 pregnant women in the Pará state [27] and when screening 116,689 pregnant women, a prevalence of 0.11% in Mato Grosso do Sul [28].

Medeiros *et al.* have tested 643 pregnant women in a prenatal unit in Curitiba, in the Paraná state (southern Brazil) and found just four reactive samples for HTLV-1, of which two were confirmed by molecular amplification [29]. It is important to mention that people of black African descent are under-represented in this state but well represented in Bahia state (northern Brazil).

Mello *et al.* found that 29 of 2766 pregnant women tested positive for HTLV-1 [30]. Contrasting results were reported by Machado Filho *et al.* who found no HTLV-1 carriers among 674 pregnant women in Manaus city, Amazon state in northern Brazil [31].

Moura *et al.* have reported that in Maceio, in the Brazilian northeast state of Alagoas, 0.2% of 54,798 pregnant women had positive HTLV-1 serology [32]. A low HTLV-1 prevalence of 0.12% was found in Fortaleza, Ceará (northeastern Brazil), among 814 pregnant women [24]. In São Luis, Maranhão state, 0.2% had antibodies to HTLV-1 and 0.15% to HTLV-2 among 2044 pregnant women [33]. Among 1033 mothers in Paraíba state who were breastfeeding, data showed an 0.68% HTLV-1 prevalence [24]. In the Rio de Janeiro metropolitan area (southeastern Brazil), Monteiro *et al.* found a 0.58% prevalence for HTLV-1 and 0.08% for HTLV-2 among 1204 pregnant women [34]. In Vitória, Espírito Santo (southeastern Brazil), 0.62% of 161 pregnant women had antibodies to HTLV-1 [35]. In Botucatu county, São Paulo state (southeastern Brazil), Olbrich Neto and Meira found one individual with HTLV-1 infection in 916 pregnant women (0.1%) [36]. Overall, Bahia state, as confirmed by several reports, has the highest HTLV-1 prevalence, independently of the population profile surveyed, and which is mainly influenced by ethnicity [11,26,30]. Therefore, the Oiapoque HTLV-1 prevalence was similar to that in Manaus, Amazonas state [31].

It is important to mention that during the period of blood collection, there was an epidemic of Chikungunya virus in the Oiapoque county [37,38], therefore possible interference in antibody detection for HTLV-1/2 cannot be formally ruled out.

In Santa Cruz do Arari, which is 582 km from Oiapoque, we have confirmed a high HTLV-1 prevalence among women of reproductive age, with five of 217 (2.3%) participants testing positive for the virus. The predominance of black African descendants living in relative isolation and consanguinity are enlisted as factors that will facilitate viral maintenance and circulation in the community, as was shown in our earlier results from 1987 [17]. Overall, we have found a 2.8% HTLV-1 prevalence in the Santa Cruz do Arari population, although none of the HTLV-1 carriers had any overt disease.

Another report by Vallinoto *et al.* has also confirmed a high HTLV-1 prevalence in the Santana do Arari population [39], a community

of black African descent located 106 km from Santa Cruz do Arari, both communities living on the Marajó Island. Workers dealing with cattle in Marajó Island move between farms on horseback in the summer, as do fishermen by boat, all attracted by the biggest lake in Santa Cruz do Arari, where people have traditionally been involved in water buffalo and fishery management, respectively. Therefore, human movement in the above-mentioned area of the Island could be an important factor in HTLV-1 transmission and its maintenance within the population.

Our previous studies in 1987 [19,40] reported the HTLV-1 *ltr* sequence circulating in Santa Cruz do Arari, which showed a minor genetic variability when compared to the HTLV-1 Japanese prototype ATK-1. Additionally, Pessôa *et al.* [41] have carried out amplification and sequencing of the HTLV-1 full-length genome from two overlapping fragments of 90 Brazilian isolates, distributed in 48 HTLV-1 asymptomatic carriers, 35 cases of HTLV-1-associated myelopathy and seven adult T-cell leukaemia/lymphoma (ATLL) patients using the Illumina paired-end methodology. Authors have concluded that 95.5% of all isolates fitted the cosmopolitan transcontinental sub-subtypes, thereby confirming our previous results [20,40]. Vallinoto *et al.* have sequenced the HTLV-1 5' *ltr* region placing the virus circulating in Santana do Arari community in the Cosmopolitan group, subgroup Transcontinental [39]. As previously mentioned, people from Santana do Arari freely move to Santa Cruz do Arari, as both communities are located on Marajó Island.

Despite a suspicion of an elevated HTLV-1 prevalence in the Oiapoque county, it was found to be very low as none of the mothers screened had antibodies to the virus. Ethnicity is not the sole factor influencing our results. As previously demonstrated, the mother's age is an important variable, with 74.5% of the pregnant and breastfeeding women participants between the age of 16 and 30 years (Table 1). It is well known that during pregnancy, mainly in the first trimester, immunological alterations allow fertilisation and zygote implantation [42]. A comparative study between HTLV-1 carriers and non-carriers among pregnant women has also shown that immunological suppression is enhanced among HTLV-1 carriers [43].

HTLV-1 prevalence among women increases with age and is mainly determined by the risk of viral sexual transmission. Vertical transmission from the mother to the infant depends on the duration of the breastfeeding period. However, in the Santa Cruz do Arari county, we have found and confirmed previous data of an elevated HTLV-1 prevalence among both women of reproductive age and men. This finding may be explained by the contribution of black African descendants in the ethnic composition of the community and its relative isolation, together with consanguinity. In the Santa Cruz do Arari county, we have found a high HTLV-1 prevalence, not only compared to that seen in the Oiapoque county, but also when compared to other counties on the same island, in Marajó and all over the country.

In conclusion, distinct HTLV-1/2 epidemiological profiles were found in two Brazilian communities located in close proximity but with different ethnic composition and behaviour. Our results confirm other reports regarding the elevated HTLV-1 prevalence among black African descendants with a high level of consanguinity in the Santa Cruz do Arari community.

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