

Geographical Clustering of Human T-Cell Lymphotropic Virus Type 1 Infection in Honduras

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Received 8 May 1995/Returned for modification 6 July 1995/Accepted 22 August 1995

Geographical clustering of human T-cell lymphotropic virus type 1 (HTLV-1) infection has been identified in the nonmestizo communities in several cities along the Atlantic coast of Honduras. Of the 2,651 serum samples tested, 122 samples were repeatedly reactive for HTLV-1 antibodies in two different enzyme immunoassays and 3 were indeterminate. These sera did not react in the HTLV-2-specific antibody tests. The presence of HTLV-1 antibodies was confirmed by HTLV-1 immunoblots or Western blots (immunoblots), and the infection was verified by the detection of HTLV-1-specific genetic sequences in the cellular DNA by PCR. Genomic DNA from the peripheral blood mononuclear cells was first tested with generic primers and probes that identified both HTLV-1 and HTLV-2. Next, all DNA samples that showed HTLV reactivity were tested by PCR with specific primers and probes that distinguished HTLV-1 sequences from those of HTLV-2. Our results indicate that only HTLV-1 infection was present in the blood of both mestizo and nonmestizo residents of 15 cities in the Republic of Honduras. The overall prevalence of HTLV-1 infection in the nonmestizo population was 8.1% (95% confidence limit, 6.6 to 9.7%). The mestizo population residing in the same geographical vicinities showed HTLV-1 antibodies in 0.5% of serum samples tested (95% confidence limit, 0.6 to 1.7%), indicating a significantly greater prevalence of HTLV-1 infection in the nonmestizo population than in the mestizo ethnic groups living in Honduras ($P = <0.0001$). Since no HTLV-2 antibody reactivity or HTLV-2-specific genetic sequences were detected by PCR with different primers and probes, it was concluded that HTLV-2 infection was not present in the Honduran population groups we tested. Our study also suggested an endemic nature for this virus because there was no difference in the prevalence rate of HTLV-1 antibodies in the nonmestizo community living in the coastal towns of Honduras between 1989 and 1993. This is the first report of HTLV-1 cluster identification in Honduras, Central America.

The human T-cell lymphotropic virus type 1 (HTLV-1) has been etiologically associated with adult T-cell leukemia, tropic spastic paraparesis, or HTLV-1-associated myelopathy (9, 14, 26, 31). An antigenically related virus, HTLV-2, which was isolated from patients with hairy cell leukemias (15, 29), has been shown to be prevalent among intravenous drug users, blood donors, Guaymi Indians, and Pygmies from Colombia, Brazil, and Africa (4–6, 8, 10, 12, 17, 19–22, 30). Although the first cluster of HTLV-1 infections was identified in patients with adult T-cell leukemia from the southern part of Japan, several studies have indicated that the prevalence of both HTLV-1 and HTLV-2 globally is higher than previously envisioned (4–6, 8, 10–12, 19–22, 25, 30). The HTLV-1- or HTLV-2-related viruses are endemic in Africa and the Caribbean basin, where they have been associated primarily with asymptomatic infections or with mild to severe neurological symptoms including TSP-HAM (4–6, 8–12, 14, 15, 17, 19–22, 25, 26, 29–31). However, the full spectrum of diseases associated with HTLV-1 and HTLV-2 infections is not completely understood.

Because of the serological cross-reactivities of HTLV-1 and -2 antibodies, most of the earlier reports were not able to distinguish between these two virus infections. With the availability of synthetic peptide-based immunoassays and the PCR methodologies, HTLV-1 and HTLV-2 infections can now be

distinguished both at the antibody level and at the genome level (3, 13, 16, 18, 23, 24, 28). Although genetically variable and prototype-related HTLV-1 and HTLV-2 strains or subtypes have been identified in several countries or regions worldwide, including South America (2, 3, 7, 11, 13, 16, 18, 22–25, 28, 30), these human retroviruses or diseases associated with them have not been reported in the Republic of Honduras. Since Honduras has strong commercial and cultural ties with the Caribbean basin, where HTLV is endemic, we undertook the present study to delineate the prevalence of HTLV-1 and HTLV-2 infections in this region. Herein we present the first report on the geographical clustering of HTLV-1 infection in different cities along the Atlantic coast of Honduras.

MATERIALS AND METHODS

Source of material. Serum samples were collected at the National University of Honduras, which is located in the capital city of Tegucigalpa, approximately 250 km away from the Atlantic coast. The Republic of Honduras encompasses 112,000 km² of the Central American region, with its main ports located along the Atlantic coast (Fig. 1). All major commerce and trade between Honduras and the rest of the world are through the major cities along the Atlantic coast. The Honduran population consists of ≈ 5 million people, primarily belonging to the ethnic group called mestizo. However, along the Atlantic coast there is a large nonmestizo population with an ancestral origin in Africa or in the Caribbean islands.

A total of 2,651 blood samples were collected during 1989 to 1993 from both mestizo and nonmestizo residents ($n = 1,341$ and 1,310, respectively) from 15 cities throughout Honduras. Sera were separated from clotted blood by centrifugation at 2,500 rpm for 10 min and stored at -20°C until tested. The sample size consisted of a large number of sera from mestizo and nonmestizo ethnic

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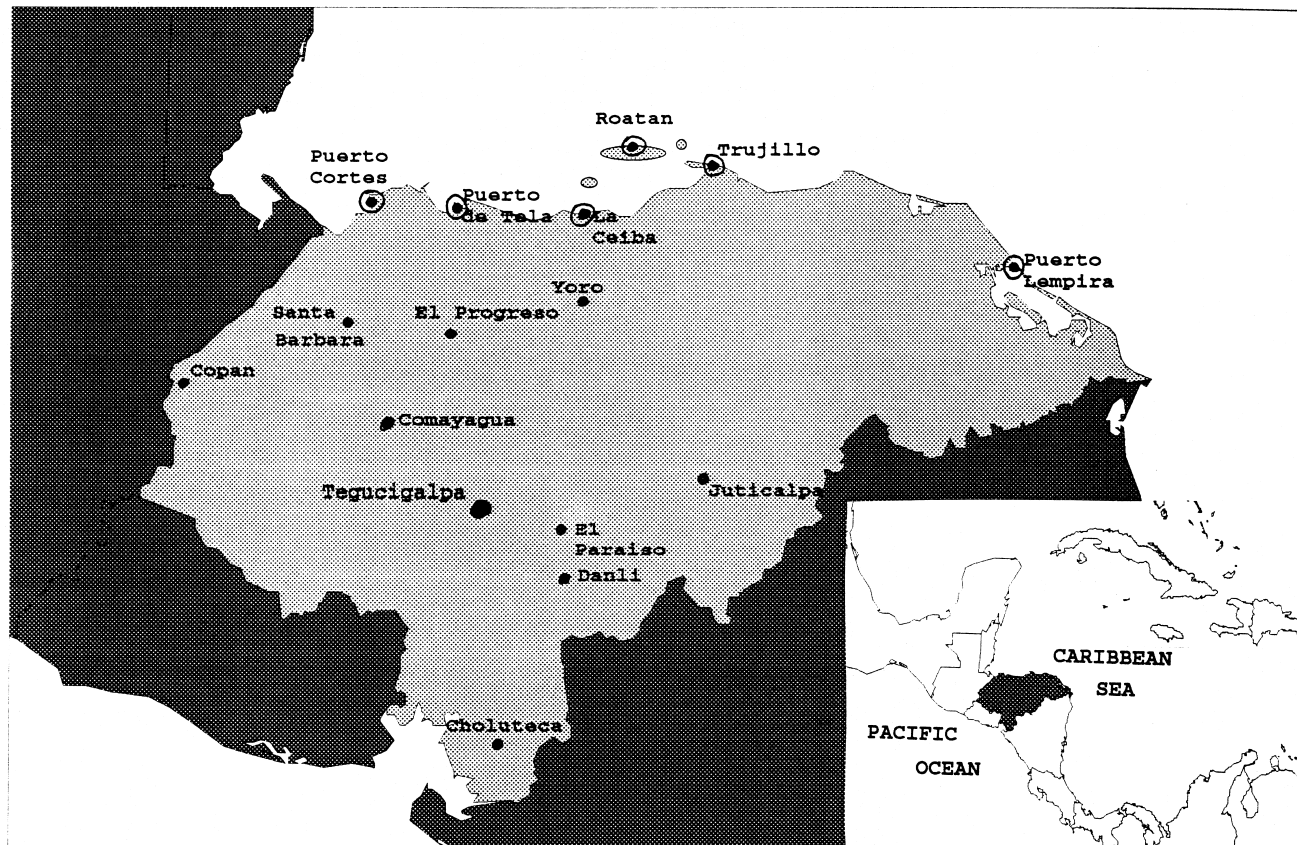


FIG. 1. Map of the Republic of Honduras showing major towns from which sera were collected. Inset on the right depicts the proximity to other Central American countries and the Caribbean.

groups ($n = 596$ and $1,267$, respectively) living in the Atlantic coastal towns of Puerto Cortes, Puerto de Tela, La Ceiba, Trujillo, Roatan, and Lempira (Tables 1 and 2). With the exception of 43 serum samples from the nonmestizo subjects who sought medical attention at the local clinic in Puerto Cortes, the main part of the country, all other samples were from volunteers who participated in the study and were recruited at their homes. None had sought any medical attention in the 6 months prior to the blood donation. Serum samples were also collected randomly from mestizo blood donors ($n = 745$) inhabiting 13 cities across the country, including Puerto Cortes, Yoro, Trujillo, Tela, Santa Barbara, El Progreso, Danli, Copan, La Ceiba, Comoyagua, Choluteca, Juticalpa, and Tegucigalpa (Table 2).

Serological testing. All 2,651 serum samples were screened at the central laboratory in Tegucigalpa, Honduras, by enzyme immunoassay (EIA) that identified both HTLV-1 and HTLV-2 antibodies, according to the instructions provided by the manufacturer (Abbott Diagnostics, Chicago, Ill.). All reactive and indeterminate samples were retested by the same EIA. The repeatedly reactive HTLV-1/2 antibody samples were confirmed for the presence of specific antibodies by HTLV-1 protein immunoblot (Western blot tests) at the Laboratory of Viral Oncology and AIDS Research, University of Southern California School of Medicine. Western blots were prepared by the use of purified HTLV-1 antigens (Organon Teknika) according to the techniques described previously (27). A positive control serum pool for HTLV-1 antibody was made from three serum samples collected from patients with adult T-cell leukemia. The negative control was a serum pool of three seronegative blood donors. Sera exhibiting bands reactive to virus-specific *gag* p24/25, p19, and *env* gp46 or gp61/68 were considered positive for HTLV-1. More than 50% of the repeatedly HTLV-1 EIA-reactive sera that were confirmed by Western blots were also selected randomly to test by the SELECT HTLV-1/2 synthetic peptide-based EIA (Coulter, Hialeah, Fla.) to distinguish HTLV-1 and HTLV-2 antibody reactivities (18, 28).

Detection of HTLV-related DNA by PCR. After the initial serologic testing was completed, a subset of 16 seronegative and 44 HTLV-1-seropositive subjects from the coastal towns of Puerto Cortes and Puerto de Tela were revisited at their homes and blood samples were collected in tubes containing acid-citrate-glucose as anticoagulant. Blood from three individuals with indeterminate Western blots was not available. The peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood by Ficoll-Hypaque gradient centrifugation

as described previously (27). Separated PBMCs were washed twice in phosphate-buffered saline, and chromosomal DNA was isolated by adding lysis buffer containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.5% Nonidet P-40, 0.5% Tween 20, and 100 μ g of proteinase K per ml and incubating at 60°C for 30 min. The proteinase K was inactivated by heating the mixture to 100°C for 10 min.

Each DNA sample was subjected to several independent rounds of PCR amplifications with different primer pairs that identified HTLV-1 and/or HTLV-2 genes separately. The PCR mixture (100 μ l) contained 2.5 mM MgCl₂, 200 nM (each) the four deoxynucleoside triphosphates, 5 U of *Taq* DNA polymerase (Boehringer Mannheim), 1 \times *Taq* buffer, and ≥ 1 μ g of PBMC DNA from test samples. The mixture was heated to 94°C for 30 s and then 53°C for 30 s and 68°C for 30 s. This procedure was repeated 35 times in a thermal cycler (Perkin-Elmer), and DNA was allowed to extend at 72°C for 10 min. Each amplicon (20 μ l) was separately hybridized with one of the four different oligonucleotide probes (depending on the primer pairs used for DNA amplification), labeled with [γ -³²P]dATP. The hybridized products were subjected to size fractionation

TABLE 1. HTLV infection in population groups living in the coastal cities of Honduras

City	No. positive/no. tested (%)		95% CL	
	Mestizo	Nonmestizo	Mestizo	Nonmestizo
Puerto Cortes ^a	1/100 (1.0)	45/486 (9.3)	0.03–5.4%	6.8–12.2%
Puerto de Tela	1/113 (0.9)	49/459 (10.7)	0.02–4.8%	8.0–13.8%
La Ceiba	0/99 (0)	5/123 (4.1)	0–3.0%	1.3–9.2%
Trujillo	0/100 (0)	3/199 (1.5)	0–2.9%	0.3–4.3%
Total	2/412 (0.5)	102/1,267 (8.1)	0.06–1.7%	6.6–9.7%

^a A total of 43 samples were collected from individuals seeking medical attention at a local hospital; 8 of 43 serum samples were positive for HTLV-1 (18.6%; CL, 8.3 to 32.8%).

TABLE 2. Prevalence of HTLV-1 infection in mestizo population

Population and location	No. tested	No. positive	% Positive	95% CL
Blood banks				
Yoro	36	1	2.8	0.07–14.2%
Trujillo	22	0	0	0–12.2%
Puerto de Tela	50	1	2.0	0.05–10.5%
El Progreso	12	0	0	0–20.8%
Danli	25	0	0	0–10.9%
Puerto Cortes	7	1	14.3	0.4–54.1%
La Ceiba	57	1	1.8	0.04–9.2%
Santa Barbara	50	1	2.0	0.05–10.5%
Copan	96	1	1.1	0.03–5.6%
Comayagua	77	0	0	0–3.8%
Tegucigalpa	186	1	0.5	0.01–2.9%
Choluteca	81	0	0	0–3.6%
Juticalpa	46	0	0	0–6.2%
Subtotal	745	7	0.9	0.4–1.9%
Residents				
Roatan	84	1	1.2	0.03–6.4%
Lempira	100	2	2.0	0.2–7.0%
Subtotal	184	3	1.6	0.3–4.7%
Total	929	10	1.1	0.5–2.0%

by electrophoresis on nondenaturing 10% polyacrylamide gels and autoradiographed (Fig. 3).

In the first amplification, the generic primers SK-43 and SK-44 were used to amplify the *tax* gene region from the proviral DNA integrated in the PBMC chromosomal DNA. The resultant amplicons were hybridized to the generic probe SK45, which identifies sequences common to both HTLV-1 and HTLV-2 (2, 3, 7, 13, 16). The HTLV reactivity was tested again in a second round of PCR with a separate aliquot of the PBMC DNA and SK110/SK111 primers from the *pol/env* region. The amplicons were hybridized separately with SK112 and SK188 probes specific for HTLV-1 and HTLV-2, respectively. These probes distinguished the two viruses. Hybridization of the SK110/SK111 amplicons with SK-115 probe was considered the control because it identified sequences common to both HTLV-1 and HTLV-2 (7, 16, 25).

To define the sensitivity and specificity of PCR assays, a number of controls were used. For HTLV-1 control, 10^{-4} μ g of DNA (0.0001 μ g) from approximately 10^2 cells from a nonproductively HTLV-1-infected cell line was used. For HTLV-2, a plasmid clone (pHB6.0) was used. To increase the specificity of detection in the test samples, approximately 100 copies of HTLV-2 were used with both SK112 and SK188 primers (Fig. 3).

Statistical analysis. Data were analyzed by various statistical programs. Exact 95% confidence limits (CL) for the prevalence rates were based on the binomial distribution and were calculated according to the methods described earlier (1).

RESULTS

Of the 2,651 serum samples tested for the presence of HTLV-related antibodies, 122 samples were repeatedly reactive by Abbott's generic EIA for HTLV-1/2 antibodies, 3 serum samples were indeterminate, and 2,526 samples were repeatedly negative. The EIA-reactive sera were negative for HTLV-2 antibodies by Coulter's SELECT HTLV-1/2 synthetic peptide assay that distinguished the two viral antibody reactivities. Thus, only HTLV-1 antibody reactivity was detected by all tests.

A total of 72 serum samples were randomly selected for the Western blot test: 62 samples were from the nonmestizo population and 10 were from the mestizo group (five blood donors from Tegucigalpa and five mestizo people living in Puerto Cortes). These samples included 67 serum samples that were repeatedly HTLV-1 antibody reactive by EIA, 2 that were negative, and 3 that were indeterminate (i.e., borderline, with optical density just above the cutoff). The presence of HTLV-1

antibodies was confirmed by Western blots in 66 of 67 EIA-seropositive samples, and one serum sample showed p24 as well as p19 reactivity with a very faint gp46-reactive band and was considered a weak positive. Two negative serum samples were consistently negative in all tests. Of the three EIA-indeterminate serum samples, one showed all HTLV-1-specific bands and was considered positive, one was negative, and one remained indeterminate by HTLV-1 Western blots, showing only p24 reactivity.

Sera from the nonmestizo ethnic group showed HTLV-1 antibodies in 8.1% of the samples tested (102 of 1,267). This reactivity ranged from 1.5 to 10.7% in apparently healthy people living in different cities along the Atlantic coast of Honduras (Table 1). Among the nonmestizo residents, 10.7% reactivity was observed in the city of Tela (49 of 459; CL, 8.0 to 13.8%), 9.3% was seen in Puerto Cortes (45 of 486; CL, 6.8 to 12.2%), 4.1% was seen in La Ceiba (5 of 123; CL, 1.3 to 9.2%), and 1.5% seroreactivity was seen for this ethnic group living in Trujillo (3 of 199; CL, 0.3 to 4.3%) (Table 1). The highest prevalence of HTLV-1 antibodies (8 of 43, or 18.6%; 95% CL, 8.3 to 32.8%) among all groups tested was in a small number of 43 nonmestizo subjects who sought medical attention at a hospital in Puerto Cortes. The clinical histories of these individuals were not available.

In contrast to the HTLV-1 seroprevalence in the healthy nonmestizo population, only 2 of 412 mestizo subjects living in the same four cities and geographical locations (Puerto Cortes, Puerto de Tela, La Ceiba, and Trujillo) showed an overall low prevalence (0.5%) of HTLV-1 antibodies ranging from 0 to 1% (Table 1). A slightly higher HTLV-1 reactivity (1.2 to 2%) was observed among the mestizo people living in the coastal towns of Roatan and Lempira (1 of 84 and 2 of 100, respectively; CL, 0.03 to 6.4 and 0.2 to 7.0%, respectively) (Table 2). Sera from the nonmestizo population in these two towns were not available for comparison.

The blood donors in Honduras are routinely screened, and the overall prevalence of HTLV-1 antibodies in the mestizo blood donors from 13 cities in Honduras was 0.9% (7 of 745; CL, 0.4 to 1.9%) (Table 2). These sera included samples from Puerto Cortes, Puerto de Tela, La Ceiba, and Trujillo. This reactivity was similar to the overall prevalence (0.9%) observed in the mestizo population including those living in the coastal cities that have a high population of nonmestizo communities. The significance of one HTLV-1 antibody-reactive serum from a mestizo blood donor residing in Puerto Cortes cannot be determined as only seven samples were tested. This sample size is too small for any statistical analysis.

The HTLV-1 seropositivity for males and females was similar in the nonmestizo population in the high-prevalence coastal areas, with an overall reactivity of 8.0% in males and 8.6% in the females. An increase in seropositivity was observed along with that in age in this group, particularly in subjects 20 years or older and living in coastal towns (Fig. 2).

Both seropositive and seronegative nonmestizo subjects from the coastal towns of Puerto Cortes and Puerto de Tela were revisited in 1993, and 60 blood samples (44 seropositive and 16 seronegative) were obtained for confirmation of the presence or absence of HTLV-related genetic sequences in the DNA and to distinguish HTLV-1 and HTLV-2 infections. Sera from these 44 individuals again showed HTLV-1 but not HTLV-2 antibody reactivity both by EIA and by Western blots. The 16 serum samples were nonreactive in both tests.

All PBMC DNA samples were initially tested by PCR, with SK43 and SK44 primers and the generic probe SK45, which hybridizes to both HTLV-1 and HTLV-2 retroviruses. DNA samples from 40 seropositive individuals hybridized to this

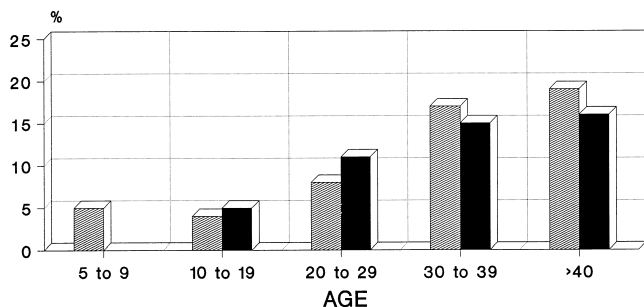


FIG. 2. The nonmestizo population was divided into males and females, and the HTLV-1 seropositivity was analyzed by age (in years) and gender. The y axis shows seroprevalence. Striped bars, females; solid bars, males.

probe. These same DNAs when amplified with SK110 and SK111 primers and separately hybridized to SK112 and SK188 probes for all 40 DNA samples showed hybridization with SK112 (HTLV-1) but not with SK188 (HTLV-2). As controls, these amplicons were also hybridized to the generic probe SK115, which recognizes both HTLV-1 and HTLV-2 viruses (Fig. 3). Four DNA samples were not analyzable because the DNA was degraded, and 16 DNA samples from HTLV-seronegative individuals were consistently negative for HTLV-1 or HTLV-2 sequences with primer pairs for both viruses and all three probes (SK-45, SK-112, and SK-188) (Fig. 3).

DISCUSSION

The study presented herein provides serologic and genetic evidence for a geographical clustering of HTLV-1 infection in Honduras with a higher prevalence in nonmestizo than in mestizo communities living in the coastal cities of Honduras. These results are statistically significant ($P < 0.0001$), and they indicate that the nonmestizo populations living in these towns are at an approximately 10 times higher risk of acquiring HTLV-1 infection than the mestizo residents in the same city. No HTLV-2 antibody or genetic sequence related to HTLV-2 was detected in this population. The mestizo ethnic groups living in the same coastal town have a much lower prevalence of HTLV-1 infection (0.5%; CL, 0.06 to 1.7%), and the overall HTLV-1 seroreactivity in this geographical area is similar to

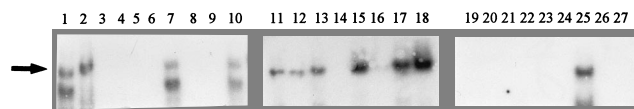


FIG. 3. Distinguishing HTLV-1 and HTLV-2 DNA sequences by PCR. DNA samples were amplified with primer pair SK110/SK111, and amplicons were hybridized to [γ - 32 P]ATP-labeled probes; SK115 hybridizes to both HTLV-1 and -2. SK112 is specific for HTLV-1, and SK188 hybridizes to HTLV-2-specific gene sequence. Lanes 1 to 4, amplicons hybridized to SK115. Lanes 5 to 7, amplicons hybridized to SK112. Lanes 8 to 10, amplicons hybridized to SK188. Lanes 11 to 18, amplicons hybridized to SK112. Lanes 19 to 27, amplicons hybridized to SK188. Lanes 3, 5, and 8, DNA from PBMCs of HTLV-1/2-seronegative donors. Lanes 1, 7, and 9, DNA from HTLV-1-infected cell line used as positive control for HTLV-1 *pol/env* genes. Note that there is no hybridization with HTLV-2-specific SK188 probe in lane 9. Lanes 2, 6, 10, and 25, plasmid (pH6B5.0) containing HTLV-2 *gag, pol*, and partial *env* was used as positive control for HTLV-2 *pol/env* genes. Note no hybridization with HTLV-1-specific SK112 probe in lane 6. Lane 4, reagent control with no DNA. Note that there is no hybridization with HTLV-1/2 generic probe SK115. Lanes 11 to 18, DNA from test subjects hybridized with HTLV-1-specific SK112 probe. Lanes 19 to 24 and 26 to 27, DNA from test subjects hybridized with HTLV-2-specific SK188 probe. No band was detected.

the national average in the mestizo blood donors (Tables 1 and 2).

The relationship between the presence of HTLV-1 antibodies and disease(s) associated with this virus infection in Honduras is not known. Although a higher prevalence of HTLV-1 antibodies in 8 of 43 (18.6%) nonmestizo subjects who attended medical clinics in one of the coastal cities (Puerto Cortes) suggests that HTLV-1 may be involved with symptomatic illness(es), no conclusion can be drawn from this study because the clinical histories of these patients are not available and the sample size is too small.

Our finding of high seroprevalence in the age group 20 to 40 years indicates that seropositivity increases with age. However, risk factors for HTLV-1 infection in these individuals are unknown. Further, the long latency period between HTLV-1 infection and the development of the virus-related symptoms would require a prospective study of a large sample size. Currently, such a study is being planned to identify the mode and risk factors involved in HTLV transmission and the incidence of HTLV-1 related diseases in Honduras.

Although a detailed survey has not been conducted to determine the rate of HTLV-1 transmission in this community, our data do not show any difference in the HTLV-1 antibody reactivity or in the rate of seroprevalence between males and females over the 4 years during which samples were collected (1989 through 1993) (Fig. 2).

The nonmestizo communities living primarily along the Atlantic coast of Honduras are descendants of a Caribbean-African population that has lived in this geographical region for approximately 500 years. This Honduran community has maintained social and commercial ties within the nonmestizo communities in the Caribbean region, and they largely mix within the same ethnic groups living in Honduras. The data presented here also support that, among the four major coastal towns, two that are closest to the Caribbean basin (Puerto Cortes and Puerto de Tela) have a higher incidence of HTLV-1 infection (9.25 to 10.7%, respectively) than the distant town of Trujillo (1.5%). Thus, it can be speculated that the close proximity of these coastal cities to the Caribbean may account for the maintenance of HTLV-1 seropositivity in the nonmestizo people living in this region.

The role of needle sharing can be ruled out because this population does not use injectable drugs. Infection by HTLV-1 resulting from the use of unsafe (unsterile) syringes and needles in the hospitals can also be ruled out because all hospitals in Honduras use disposable needles and syringes. Since none of the subjects tested had received any blood transfusion, the HTLV-1 seropositivity cannot be related to this potential risk factor. These observations suggest that the high prevalence rate of HTLV-1 infection in the nonmestizo communities living in the coastal towns of Honduras may be related to certain social and/or sexual practices or to particular genetic predilections of these individuals.

At the present time, no data on the incidence of HTLV-1-related diseases in Honduras are available. Because nonmestizo and mestizo populations have had a limited interaction, the opportunity exists to study the natural history of disease(s) caused by HTLV-1 and determine the transmission rate in a community of people with endemic infection. To our knowledge, this is the first demonstration of the high prevalence of HTLV-1 infection in the nonmestizo communities of the coastal towns of Honduras.

ACKNOWLEDGMENTS

We thank Zhijuan Sheng for the statistical analysis of the data and Laura Garibay for the preparation of the manuscript.

This work was supported in part by the Fulbright Scholarship to Ivette L. de Rivera to work at the Laboratory of Viral Oncology and AIDS Research at USC, Los Angeles, which is also supported by grants from the National Institutes of Health (AI27673 and T32-A107383).

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