

Sensitivity of Two Enzyme-linked Immunosorbent Assay Tests in Relation to Western Blot in Detecting Human T-Cell Lymphotropic Virus Types I and II Infection among HIV-1 Infected Patients from São Paulo, Brazil

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We investigated the presence of human T-cell lymphotropic virus types I and II (HTLV-I and HTLV-II) infections, first searching for specific antibodies in 553 serum samples obtained from HIV-1-infected patients from São Paulo, Brazil. Sera were screened using two enzyme-linked immunosorbent assays (ELISAs): the ELISA-EM (ELISA HTLV-I/II, EMBRABIO, BR), which contains HTLV-I and HTLV-II lysates, and the ELISA-DB [ELISA HTLV-I/II, Diagnostic Biotechnology (DB), Singapore], which contains HTLV-I lysate, and HTLV-I and HTLV-II recombinant env proteins (MTA-1 and K55, respectively). Serum samples showing two positive and/or borderline results were confirmed by Western blot (WB 2.3, DB), which discriminates HTLV-I from HTLV-II. WB analyses disclosed 22 cases (4.0%) of HTLV-I and 34 (6.1%) of HTLV-II seroreactivity; 24 sera had indeterminate antibody profile (4.3%) and 2 specimens showed reactivity to both

MTA-1 and K55 env proteins. Using stringent WB criteria and analyzing the population according to risk factors, the prevalence rates of HTLV-I and HTLV-II infections were 11.2% and 16.8% in IV drug users, 3.4% and 5.5% in heterosexual individuals, and 1.4% and 2.2% in homosexual/bisexual men, respectively. A comparison of ELISA and WB results disclosed that both ELISAs were highly sensitive in detecting HTLV-I antibodies, whereas the ELISA-DB showed 82% sensitivity and the ELISA-EM 100% sensitivity in detecting HTLV-II antibodies. PCR analyses conducted on 37 representative cells samples confirmed the presence of HTLV proviral DNA in the majority of concordant serological cases, except in one, which was HTLV-I infected and seroreacted with K55 protein of HTLV-II. Indeed, after PCR, one case of HTLV-I infection and HTLV-II coinfection, and 30% of WB-seroindeterminate or inconclusive cases infected with HTLV-II could be detected. Our data stress high prevalences of both HTLV-I and HTLV-II infections in HIV-1 coinfecting i.v. drug users from São Paulo, and suggests that ELISA kits containing only K55 protein as the HTLV-II-specific antigen, may not have the appropriate sensitivity for the detection of HTLV-II infection in this geographic region, pointing out the need of improved screening tests to be used in Brazil.

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INTRODUCTION

Infections with human T-cell lymphotropic virus type I (HTLV-I) and HTLV-II are widely distributed among recipients of multiple blood transfusions, i.v. drug users (IVDU), female prostitutes, and patients attending sexually transmitted disease clinics (Kaplan and Khabbaz 1993). HTLV-I is the etiologic agent of adult T-cell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis. HTLV-II has not yet been etiologically linked to any particular disease, although rare, isolated cases of hairy cell leukemia and a neurological syndrome resembling HTLV-I-associated myelopathy have recently been reported (Hollsbury et al. 1993; Kaplan and Khabbaz 1993). Infection with HTLV-I is endemic in certain geographic areas, including Japan, Melanesia, the Caribbean Islands, and parts of Africa and Brazil. HTLV-II is now recognized as a highly prevalent infection among IVDU and as an endemic retrovirus of several indigenous Amerindian populations (Kaplan and Khabbaz 1993).

Both HTLV-I and HTLV-II are transmitted in a cell-associated manner, with major routes of transmission being sexual (predominantly male to female), mother to child (breast-fed infants of seropositive mothers), and parenteral injection of contaminated blood (blood transfusion or i.v. drug use) (Kaplan and Khabbaz 1993). Because of the risk of blood-borne infections associated with HTLVs, screening of volunteer blood donors was implemented first in Japan in 1986 and then in USA in 1988 (Busch et al. 1994; Kaplan and Khabbaz 1993). Donor screening is also performed in Canada, Trinidad and Tobago, the French West Indies, France, the Netherlands, and since 1993, in Brazil (Brasil-Ministério da Saúde 1995).

Many reports indicate that HTLVs are present in the same populations at risk of AIDS; indeed, studies of HIV-1-seropositive subjects in Europe, USA, and Brazil disclosed coinfection with HTLVs varying from 2 to 11% of cases (Caterino-de-Araujo et al. 1994; Lentino et al. 1991; Robert-Guroff et al. 1986; Tedder et al. 1984). When the risk factor for acquiring retroviruses was drug use, this rate increased up to 20% among Brazilian AIDS patients (Barbosa de Carvalho et al. 1996; Caterino-de-Araujo et al. 1994; Moreira et al. 1993).

The ability to make accurate and reliable diagnoses of HTLV-I and HTLV-II infections is essential both for ensuring the safety of blood and for understanding their roles in human disease. The discrimination of HTLV-I and HTLV-II infections is important for epidemiological and public health studies, and to study their cofactorial role in AIDS progression.

The overall structural similarity between these two retroviruses and the identity of much of the primary amino acid sequence suggest antigenic homology between HTLV-I and HTLV-II. This homology is responsible for the cross-reactivity in serological assays.

Serologic confirmation of HTLV-I and -II infections requires the demonstration of antibodies to gag (p24) and env (gp46 and/or gp68) proteins (CDCP and USPHS Working Group 1993). Specimens reacting with any of the protein bands, but not satisfying the criteria given above, are designated "seroindeterminate."

The sensitivities of Western blot assays (WB) for the detection of antibodies to envelope proteins of HTLVs have been substantially enhanced by the addition of the recombinant transmembrane protein r21e, which can detect antibodies in all HTLV-I and -II infected individuals (Buchner et al. 1992); nevertheless, this resulted in an increased proportion of false-positive results (Lal et al. 1992). To overcome this handicap, specific epitope within the transmembrane envelope protein of HTLV-I, designated GD21-I, was incorporated on WB in addition to recombinant external glycoprotein (rgp46) of both HTLV-I (MTA-1) and HTLV-II (K55) (Brodine et al. 1993; Varma et al. 1995). However, because of genetic heterogeneity among various isolates, no reactivity to MTA-1 and K55 were documented in certain geographic areas, especially in Tropical regions including Brazil (Caterino-de-Araujo et al. 1994; Garin et al. 1994).

Because of the fact that we previously demonstrated a high prevalence of HTLV-I and -II infections in IVDU with AIDS living in São Paulo, and pointed out the deficiency in serological screening tests to confirm and discriminate HTLV-I and -II infections (Caterino-de-Araujo et al. 1994), we decided to extend and confirm these data by analyzing blood samples obtained from AIDS patients living in the same geographic area. Using serological assays usually employed in Brazil, we determine the sensitivity of the ELISA tests in relation to the confirmatory WB 2.3.

To confirm the presence of HTLV proviral DNA, polymerase chain reaction (PCR) analyses were conducted on DNA extracted from cells obtained from 37 HTLV-seropositive patients.

MATERIALS AND METHODS

Study Population

From June to July 1994, serum samples obtained from HIV-1-infected individuals referring to the Instituto de Infectologia Emílio Ribas of São Paulo

(Brazil) (IIER) for medical assistance were screened for HTLV-I/II infections. Subjects with any HTLV seroreactivity were recalled, and after informed consent, a second blood sample was collected for further serological and molecular analyses. Clinical status and risk factors for retroviroses were obtained from medical records and by interview.

Serological Analyses

Sera were screened using two commercially available ELISA tests: the ELISA-EM (HBK 454 Hemobio anti-HTLV-I and -II, EMBRABIO, BR), which contains HTLV-I and HTLV-II whole virus lysates (lot EEA01I6, 1994), and the ELISA-DB (ELISA-HTLV-I/II, Diagnostic Biotechnology, Singapore), which consists of HTLV-I virus lysate and HTLV-I-specific (MTA-1) and HTLV-II-specific (K55) recombinant envelope proteins (lot BE0800876, 1994). Criteria of positivity were according to the manufacturer's instructions; however, to reduce false-negative results, a 20% reduced cutoff value was used in both ELISA assays (BL-).

Serum samples showing twice positive and/or borderline results in either or both ELISAs were tested by WB (HTLV Blot 2.3, Diagnostic Biotechnology). The antibody profile was defined according to the following criteria: sera reactive to at least one gag protein (p19 or p24) and two env proteins (gp46, rgp46-I, and rgp21) were considered HTLV-I positive; reactivities to at least the p24 gag protein, rgp21, and rgp46-II were considered HTLV-II positive; sera reactive to p19 or p24 and rgp21 were classified as HTLV-I/II; sera reactive to only gag or env proteins were denoted inconclusive; sera exhibiting no reactivity were considered negative.

PCR and Nested PCR

Amplifications were carried out as previously described (Calabrò et al. 1993) using primers specific for tax and env regions of both HTLV-I and HTLV-II genomes. For the tax region of HTLV-II, a segment of 1253 bp was amplified using primer pairs that anneal to nucleotides 7077-7094 and 8330-8313 of MoT isolate, and for a tax segment of 221 bp common to HTLV-I and HTLV-II, nucleotides 7359-7380 and 7570-7549 of ATK isolate, and nucleotides 7249-7270 and 7460-7439 of the MoT isolate, respectively. The entire env regions (1.4 kb) of HTLV-I and HTLV-II (nucleotides 5202-5219 and 6668-6651 of ATK isolate and nucleotides 5180-5197 and 6642-6625 of MoT isolate) were also amplified.

Nested PCR was performed as described earlier using a set of primers that allows the amplification of an internal 960 bp region of the tax gene of HTLV-II (nucleotides 7364-7377 and 8330-8313). A nested set

of primers for HTLV-I env region was also used (nucleotides 5229-5247 and 6649-6632 of ATK). An internal 762 bp region of the envelope gene of both HTLV-I and HTLV-II (nucleotides 5581-5601 and 6343-6323 of ATK, and nucleotides 5547-5567 and 6309-6289 of MoT; Calabrò et al. 1993) were amplified.

A gel electrophoresis analysis of PCR and nested PCR products confirmed and typed HTLV infection.

RESULTS

We studied 358 (64.7%) men and 195 (35.3%) women, ranging in age from 13 to 65 years; mean age was 32 years. Based on their risk factors for acquiring retrovirus infection, the patients could be classified into groups: 89 (16.1%) were IVDU (65 men and 24 women, mean age 29 years), 6 (1.1%) were exposed to blood transfusions (2 men and 4 women, mean age 31 years), 236 (42.7%) mentioned heterosexual contact with multiple partners, AIDS patients, or IVDU (96 men and 140 women, mean age 33 years), 139 (25.1%) were homosexual/bisexual men (mean age, 33 years), and 83 (15.0%) the risk factors were unknown (56 men and 27 women, mean age 32 years). All individuals were HIV-1 infected, and 353 (64.5%) had AIDS according to clinical and laboratorial evaluations; in 50 (9.1%) cases the stage of HIV-1 infection was not available.

The preliminary serological screening conducted using the ELISA-EM and ELISA-DB disclosed 87 cases of HTLV seroreactivity (sera tested twice positive and/or borderline in either or both ELISAs). Of these, 82 samples resulted positive or indeterminate on WB analyses (Table 1); 22 cases (4.0%) were found to be HTLV-I-positive, 34 (6.1%) were HTLV-II-positive, and 24 (4.3%) showed indeterminate antibody profiles (9 classified as HTLV-I/II and 15 as inconclusive according to WB criteria described in Materials and Methods). Reactivity to both HTLV-I and HTLV-II specific recombinant env proteins was demonstrated in two cases.

Using WB as the "gold standard" in defining HTLV-I and -II infections, the results demonstrated that both ELISAs are highly sensitive in detecting HTLV-I-specific antibodies. On the other hand, the ELISA-EM was more accurate than the ELISA-DB for detecting HTLV-II-specific antibodies (100% vs. 82% sensitivity, respectively), with 95% confidence intervals (CIs): 78.8–85.2%. To reduce false-negative results, a 20% reduced cut-off value was used (see B1⁻ in Table 1). This modification increased the sensitivity of HTLV-II antibody detection by ELISA-DB (88%, 95% CI: 85.3–90.7%); however, four cases that were classified as HTLV-II positive by WB remained negative with the reduced cutoff.

TABLE 1 Comparison of the HTLV Serological Results Obtained in a Population of 553 HIV-1-infected Individuals

WB Results	No.	ELISA-EM				ELISA-DB			
		Pos	B1 ⁺	B1 ⁻	Neg	Pos	B1 ⁺	B1 ⁻	Neg
HTLV-I	22	22	0	0	0	22	0	0	0
HTLV-II	34	33	1	0	0	27	1	2	4
HTLV-I and -II	2	2	0	0	0	2	0	0	0
Indeterminate	24	19	1	2	2	5	6	0	13
Nonreactive	471	5	0	0	466	1	0	0	470
Total	553	81	2	2	468	57	7	2	487

WB findings are compared to data obtained by means of the ELISA-EM and ELISA-DB (see Materials and Methods). Using a 20% reduced cutoff value (B1⁻), the ELISA-DB still fails to detect four HTLV-II-seropositive sera, whereas the ELISA-EM, which contains both HTLV-I and -II whole virus lysates, shows 100% sensitivity in detecting both HTLV-I and -II-specific antibodies. B1⁺, borderline values. B1⁻, 20% reduced cutoff.

Cases presenting discordant results in the serological screening were reexamined 2 months later; results of the two sequential samples are reported in Table 2. The second serological analysis confirmed the lower sensitivity of the ELISA-DB in detecting HTLV-II-specific antibodies. WB antibody profiles of the second specimens are shown in Figure 1.

Considering only the samples with defined result on WB 2.3, we identified 22 cases of HTLV-I infection (4.0%, 95% CI: 2.4–5.6%) and 34 cases of HTLV-II (6.1%, 95% CI: 4.1–8.1%) in a total of 553 HIV-1-infected subjects; Table 3 presents the results obtained according to risk factors and stage of HIV-1 disease, showing that HIV-1/HTLVs coinfection was mostly associated with i.v. drug abuse and AIDS stage of HIV-1 infection. The high prevalence of 11.2% (95% CI: 8.5–13.9%) for HTLV-I and 16.8% (95% CI: 13.7–19.9%) for HTLV-II among IVUDU contrasts significantly with the prevalences of 1.4% for

HTLV-I and 2.2% for HTLV-II observed among homosexual/bisexual men. Indeed, significant low prevalence rates were also observed among HIV-1/HTLV coinfecting men and women referring heterosexual contacts when compared with rates obtained among IVUDU.

To confirm HTLV serological diagnosis, the 82 individuals with positive or indeterminate WB 2.3 profiles were recalled for further serological and molecular analyses; 37 responded, and fortunately, in this series we had samples representative of all WB patterns prior obtained.

According to high stringency criteria for HTLV seropositivity, 8 of the 37 sera were found to be HTLV-I-positive, 19 were HTLV-II-positive, and 2 were found to be positive for both HTLV-I and -II infections; 8 samples showed an undetermined antibody profile (3 classified as HTLV-I/II and 5 as inconclusive).

TABLE 2 Summary of Discordant Serological Results Obtained in Two Sequential Samples of 13 Patients

Patient	ELISA-EM	ELISA-DB	WB 2.3	ELISA-DB	WB 2.3
	First Sample			Second Sample	
27	++/++	B1+/+	Ind	B1+	HTLV-II
36	+/+	-/+	HTLV-II	-	Ind
51	++/++	B1+/++	Ind	-	Ind
55	++/++	-/-	Ind	-	Ind
85	++/++	B1+/+	HTLV-II	-	HTLV-II
121	++/++	++/++	Ind	B1-	Ind
173	++/++	B1-/-	HTLV-II	-	HTLV-II
261	B1+/ND	++/++	Ind	B1-	Ind
288	B1-/ND	++/++	Ind	+	Ind
294	++/++	-/++	Ind	-	Ind
315	+/+	+/++	Ind	-	Ind
376	++/B1-	-/+	HTLV-II	-	HTLV-II
471	++/++	-/+	Ind	-	Ind

ND, not done; B1+, borderline value; B1-, 20% reduced cutoff value; -, negative; ++, at least twice the cutoff value; Ind, indeterminate.

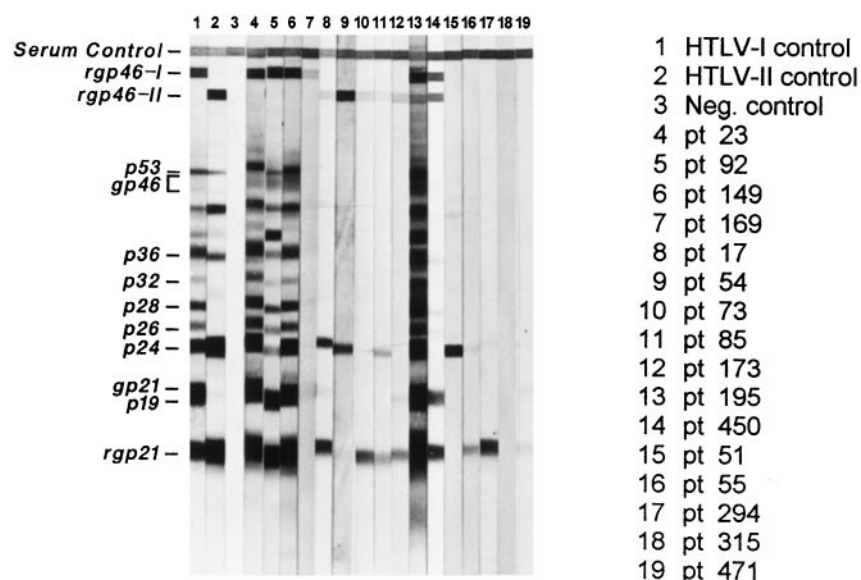


FIGURE 1 WB of the second serum specimen obtained from patients with discordant serological results (see also Table 2).

PCR analyses confirmed and typed the HTLV infection in 25 cases (Table 4). HTLV-I-specific sequences were detected in 8 patients (5 of 8 HTLV-I-seropositive cases, 1 of 19 HTLV-II-seropositive patients, and in both HTLV-I and -II-seropositive patients); HTLV-II specific sequences were amplified in 18 cases (14 of 19 HTLV-II-seropositive patients, 1 of 2 HTLV-I and -II-seropositive patients, 2 of 3 HTLV-I/II-seropositive patients, and 1 of 5 inconclusive cases). It is important to note that patient 450 resulted double infected. Figure 2 shows a gel electrophoresis analysis of nested PCR products of some representative HTLV-II samples.

DISCUSSION

The development of serologic assays that can reliably differentiate between infections with HTLV-I and -II simplified seroepidemiologic studies of HTLV-infected populations have been studied (Buchner et al. 1992; Gallo et al. 1991; Kline et al. 1994; Lipka et al. 1992; Rudolph and Lal 1993; Varma et al. 1995).

Brazil appears in the rank of endemic areas mostly for HTLV-II infection, with prevalence rates up to 30% among the Amerindian population from the Amazon region (Black et al. 1994; Gabbai et al. 1993; Ishak et al. 1995).

In Salvador, Bahia, high prevalences of HTLV-I infection in healthy subjects and AIDS patients were described (Moreira et al. 1993). This probably reflects the ethnic background of the population (African origin) and the behavioral risk factors for acquiring retroviruses. In agreement, HTLV-I and HTLV-II are present in other geographic regions from Brazil in the same populations at risk of AIDS, disclosing prevalence rates varying from 2 to 25% (Barbosa de

Carvalho et al. 1996; Caterino-de-Araujo et al. 1994; Cortes et al. 1989; Gabbai et al. 1993).

We previously demonstrated a high prevalence of HTLV-I and -II infections in IVDU with AIDS from São Paulo, and therefore pointed out the deficiency in serological screening tests to confirm and discriminate HTLV-I and -II infections in this geographic region (Caterino-de-Araujo et al. 1994). Using stringent criteria for HTLV seropositivity, we confirmed 7.9% of HTLV-I and 5.4% of HTLV-II infections in HIV-1-coinfected patients attending the Instituto de Infectologia Emílio Ribas, São Paulo, SP, Brazil, in 1992. Indeed, when the risk factors were analyzed, a close association between IVDU and HTLV-I and HTLV-II infection (15.3% and 11.1%, respectively, versus 0.9% and 0.4% in homosexual/bisexual men) was detected.

Extending these data 2 years later, and using a resembling population, we could identify prevalences of 4.0% for HTLV-I and 6.1% for HTLV-II. The high prevalences of 11.2% for HTLV-I and 16.8% for HTLV-II among IVDU contrast with the prevalences of 1.4% and 2.2%, respectively, observed among homosexual/bisexual men (Table 3). The apparent increase in the rate of HTLV-II infection (11.1% in 1992 and 16.8% later on) among Brazilian IVDUs prompted us to question whether the virus was rapidly spreading in the IVDU population or, alternatively, if changes in diagnostic methods yielded a higher sensitivity for HTLV-II detection. The last hypothesis seems more reasonable because several HTLV-seronegative samples (ELISA-DB negative or borderline) were confirmed as HTLV-II infected by using PCR analyses, despite the WB 2.3 interpretation (Table 4).

TABLE 3 Prevalence of HTLV-I and HTLV-II Infections According to Risk Factors and Stage of HIV-1 Disease

Risk Factor	Group	No. Tested	HTLV-I Infected (%)	HTLV-II Infected (%)
IVDU	Men with AIDS	54	9	12
	Men without AIDS	11	0	2
	Women with AIDS	15	0	1
	Women without AIDS	7	1	0
	Women ^a	2	0	0
	Subtotal	89	10 (11.2)	15 (16.8)
Blood transfusion	Men with AIDS	1	0	0
	Men without AIDS	1	0	0
	Women with AIDS	3	0	0
	Women without AIDS	1	0	0
	Subtotal	6	0	0
Heterosexual contact	Men with AIDS	61	6	3
	Men without AIDS	27	0	2
	Women with AIDS	91	1	6
	Women without AIDS	34	1	1
	Men ^a	8	0	1
	Women ^a	15	0	0
	Subtotal	236	8 (3.4)	13 (5.5)
Homosexual/bisexual contact	Men with AIDS	87	1	2
	Men without AIDS	46	1	1
	Men ^a	6	0	0
	Subtotal	139	2 (1.4)	3 (2.2)
Unknown	Men with AIDS	31	1	2
	Men without AIDS	13	0	0
	Women with AIDS	10	1	0
	Women without AIDS	10	0	1
	Men ^a	12	0	0
	Women ^a	7	0	0
	Subtotal	83	2 (2.4)	3 (3.6)
Total		553	22 (4.0)	34 (6.1)

^a Group not available.

In the present study, even when a 20% reduced cutoff value was utilized in ELISA determinations, the sensitivity of HTLV-II-antibody detection increased by 5.9%, but continued lacking sensitivity for screening four cases of HTLV-II infection (Table 1). These results are in accordance with those reported by Weiss (1993) and Soriano et al. (1995), and point out that HTLV seroprevalence studies should be performed using screening assays that use antigens from both HTLV-I and HTLV-II viruses.

Although first-generation ELISAs lack specificity in identifying all HTLV sera, the use of both viral lysates seems to be more sensitive in detecting all Brazilian samples (see Table 1, ELISA-EM), contrasting with the ELISA that contain only the two specific recombinant antigens, MTA-1 from HTLV-I and K55 from HTLV-II (see Table 1, ELISA-DB).

Despite the fact that an overestimated HTLV infection may occur using less stringent criteria of HTLV seropositivity, we believe that in endemic areas and/or with high-risk groups, all samples that

resulted HTLV seroindeterminate could be considered as potentially infected.

Reduced cutoff values were also used by Black et al. (1994), Hjelle et al. (1994), and Rios et al. (1994) to increase the sensitivity for HTLV-II antibody detection. On the other hand, Gallo et al. (1991, 1994a) have been standing out the use of IFA as an alternative method for detecting HTLV-II weakly seroreactive samples.

Missing in HTLV-I diagnosis has also been reported; Bonis et al. (1994), studying the prevalence of HTLV infection in Burundi (East Africa), observed a low infection rate of HTLV-I, and a lack of reactivity to the HTLV-I immunodominant envelope epitope (MTA-1), analyzing sera samples on WB 2.3. They commented that the results may indicate an antigenic variation in the crucial amino acids required for antibody binding, or represent variant forms of HTLV-I.

Garin et al. (1994) showed that in a high viral endemic area of HTLV-I/II infection in Africa, only

TABLE 4 Molecular Confirmation of HTLV-I and/or HTLV-II Coinfection among 37 HIV-1-Infected Patients

Characteristics of Patient	Serological Screening					PCR Analyses	
Patient/Age/Gender/ CDC Class/Risk Factor	ELISA-EM	ELISA-DB	WB 2.3 HTLV-I	WB 2.3 HTLV-II	WB 2.3 Indeterminate	HTLV-I	HTLV-II
23/24/M/IV/IVDU	+/+	+/+	+			+	—
92/24/F/II/IVDU	+/+	+/+	+			+	—
137/36/M/IV/Hete	+/+	+/+	+			+	—
149/35/M/II/Homo	+/+	+/+	+			+	—
169/32/M/IV/Hete	+/+	+/+	+			—	—
285/44/F/IV/IVDU	+/+	+/+	+			+	—
438/38/M/IV/IVDU	+/+	+/+	+			—	—
441/39/M/IV/IVDU	+/+	+/+	+			—	—
17/31/M/IV/IVDU	+/+	+/+		+		—	+
27/32/M/II/IVDU/Bi	+/+	B ⁺ /B ⁺		+		—	+
36/31/M/IV/Hete	+/+	—/—		+		—	—
54/33/M/IV/IVDU	+/+	+/+		+		—	+
73/35/M/IV/Homo	+/+	+/+		+		—	—
85/26/M/IV/IVDU	+/+	B/—		+		—	+
120/25/M/IV/Hete	+/+	+/+		+		—	+
173/19/M/IV/IVDU	+/+	B [—] /—		+		+	—
258/28/M/IV/IVDU	+/+	+/+		+		—	+
262/36/M/IV/IVDU	+/+	+/+		+		—	+
281/29/M/IV/IVDU	+/+	+/+		+		—	+
305/22/F/IV/Hete	+/+	+/+		+		—	+
320/30/F/II/NA	+/+	+/+		+		—	+
334/38/M/IV/Hete	+/+	+/+		+		—	—
376/22/M/IV/IVDU	+/B [—]	—/—		+		—	+
419/33/F/IV/p.IVDU	+/+	+/+		+		—	—
510/26/M/II/IVDU	+/+	+/+		+		—	+
540/37/M/IV/IVDU	+/+	+/+		+		—	+
542/23/M/IV/IVDU	+/+	+/+		+		—	+
195/37/F/II/NA	+/+	+/+	+	+		+	—
450/28/M/IV/IVDU	+/+	+/+	+	+		+	+
55/26/M/IV/IVDU	+/+	—/—			p24,gp21	—	+
294/27/M/IV/IVDU	++/++	—/—			p24,gp21	—	—
471/28/M/IV/IVDU	++/++	—/—			p24,gp21	—	+
51/18/F/IV/Hete	+/+	—/—			p24	—	—
121/23/F/II/Hete	+/+	+/B—			p28,p19	—	—
261/34/F/II/p.AIDS	B+/ND	+/B—			gp46-I,II	—	—
288/28/M/IV/Bise	B—/ND	+/+			gp46-I,II	—	—
315/27/F/IV/p.IVDU	+/+	+/—			gp21,gp46-I,II	—	+

Age: years; gender: M = male, F = female; CDC classification system (1993); risk factors: IVDU = i.v. drug user; p.IVDU = partner of IVDU; hete = heterosexual; homo = homosexual; bise = bisexual; NA = not available; ND: not done. Criteria for HTLV serological screening and PCR analyses are specified in Materials and Methods.

15% of cases that resulted WB seroindeterminate were confirmed infected by HTLV-I when PCR analysis was conducted. Sixty percent of these cases were ELISA negative. The authors also discussed that in areas where the HTLV-I/II endemicity is rare or absent, such as Western Europe or the USA, WB-indeterminate sera exhibit few and faint bands, and most if not all of these individuals are HTLV-I PCR negative. In contrast, in some high endemic areas for HTLV-I of intertropical Africa, a large proportion of

indeterminate WB sera exhibit multiple band patterns, often intense, and some of these individuals are HTLV-I PCR positive.

The same explanation may be used in the present study for HTLV-II diagnosis; we detected HTLV-II true infection in two of three HTLV-I/II seropositive cases, and in 25% of WB-inconclusive cases (see results of PCR in Table 4).

Gessain et al. (1995) proposed for tropical areas the inclusion of other tests like RIPA, ELISA using

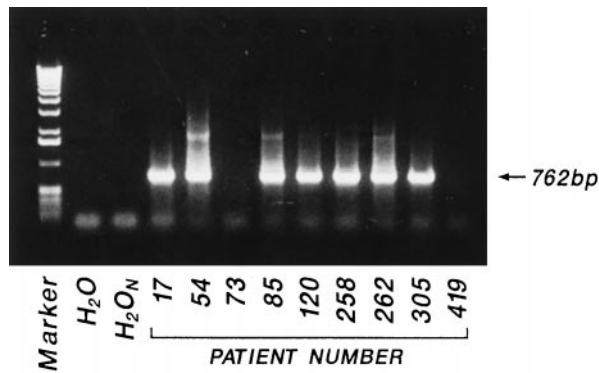


FIGURE 2 Nested PCR analysis of some HTLV-II-seroreactive specimens. Nested PCR was performed using one-twentieth of the first-round product. Specific 762-bp bands were amplified from patients 17, 54, 85, 120, 258, 262, and 305. H₂O: water control; H₂O_N: nested water control; Marker: 1-kb DNA ladder.

specific synthetic peptides, IFA, and PCR for diagnostic criteria for HTLV-I.

Defer et al. (1995) showed that they were capable of discriminating between false- and true-positive results of HTLV-I or HTLV-II infections, in the absence of MTA-1 or K55 reactivity, when rgp21 associated with one gag band (p19 or p24) was detected on WB analysis. In the absence of rgp21, PCR and/or RIPA were not required to confirm HTLV-I/II infection. Our results confirm this proposition (Table 4) and emphasize the use of ELISA-sensitive tests for screening Brazilian samples, or alternatively, IFA as suggested by Casseb et al. (1997).

Little data are available on the frequency of the WB-positive serologic status of HTLV-I ELISA-negative sera in high HTLV-I endemic areas (Garin et al. 1994; Gessain et al. 1995). In the present study, we

detected high sensitivity of both ELISAs in detecting HTLV-I antibodies using WB as gold standard (Table 1). Of interest was the fact that in one case of HTLV-II seropositivity, HTLV-I infection was confirmed by PCR analysis (Table 4, patient 173); indeed, one case of HTLV-I and HTLV-II dual infection between two dual seropositive cases was detected (Table 4, patient 450).

We could not explain these discrepant results, but the similar dual WB seroreactivity in HTLV-II-infected patients was described by Gallo et al. (1994a); they also showed specific seroreactivity to rgp46-I in HTLV-II-infected cases.

The wrong classification by WB 2.3 described by Gallo et al. (1994a) and by us raises the possibility of the presence of HTLV variants circulating in several parts of the world having significant divergent env proteins. Molecular approaches that detect amino acid changes in env regions of HTLV-I and HTLV-II isolates from different geographic regions could be encouraged to solve this puzzle. The inclusion of other synthetic peptides on serological tests used for HTLV screening could be taken in account.

Finally, the present study confirms high prevalences of both HTLV-I and HTLV-II infections mainly in HIV-1-coinfected IDU from São Paulo, Brazil, and once again points out the need for improved or alternative screening tests to be used in this country.

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