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HIV and HTLV-I Antibody Studies: Pregnant Women in the 1960s, Patients with AIDS, Homosexuals, and Individuals with Tropical Spastic Paraparesis

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To investigate the possible occurrence of human immunodeficiency virus (HIV) or human T-cell lymphotropic virus, type I (HTLV-I) infections in the United States prior to 1979-1981, when acquired immune deficiency syndrome (AIDS) was first recognized, we tested sera from 310 pregnant women who participated in the Collaborative Perinatal Project during the period 1959-1964 for HIV and HTLV-I antibody. These samples included sera from 53 pregnant women who were intravenous drug users. The remainder were from women who had cervical epithelial abnormalities, who developed cervical carcinomas, who had had children with erythroblastosis fetalis, who had had children that developed malignant neoplasms early in life, or normal pregnant women. None of the 310 women had confirmed HIV or HTLV-I antibody. The rate of false-positive reactions with the HIV enzyme-linked immunosorbent assay (ELISA) antibody test in these long-frozen samples was similar to that observed in fresh sera.

HIV antibody was detected in homosexual patients with AIDS; HTLV-I antibody was not detected in any of these sera. HTLV-I antibody was detected in 17 of 20 patients with tropical spastic paraparesis (TSP) and in two of seven patients with other neurological diseases diagnosed as transverse myelopathy and multiple sclerosis, and in none of nine normal controls; HIV antibody was not detected in any of these sera patients. Thus, we conclude that there was no serological evidence of infection with HIV or HTLV-I in the pregnant women studied; however, HIV antibody was present in all AIDS patients tested, and HTLV-I antibody was found in the majority of patients with TSP.

INTRODUCTION

The retrovirus human immunodeficiency virus (HIV) has been associated with acquired immune deficiency syndrome (AIDS) [1,2]. AIDS was first identified in 1981 and since that time the number of patients has increased dramatically. There have now been over 30,000 reported cases, more than half of whom have died. In the

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Abbreviations: AIDS: acquired immune deficiency syndrome HIV ELISA: HIV enzyme-linked immunosorbent assay HIV: human immunodeficiency virus HTLV-I: human T-cell lymphotropic virus, type I HIV IFA: HIV indirect immunofluorescent assay TSP: tropical spastic paraparesis

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United States the disease occurs primarily among homosexual and bisexual men. Other risk groups include drug users, hemophiliacs, transfusion recipients, and small numbers of heterosexual men, women, and children. In some areas, over 30 percent of the intravenous drug users have HIV antibody. The origin of the disease remains controversial. There are some indications that infections occurred in Africa for several years at least before the disease was recognized in the United States.

The retrovirus human T-cell lymphotropic virus, type I (HTLV-I) has been associated with adult T-cell leukemia and tropical spastic paraparesis (TSP) [3,4,5,6,7]. Adult T-cell leukemia associated with HTLV-I was initially identified in Japan and subsequently in other parts of the world. TSP is a slowly progressive myelopathy which affects primarily the pyramidal tracts bilaterally and symmetrically, affecting mainly the lower extremities. This disease is manifested by difficult walking, spasticity, hyperflexion, and extensor plantar response. There is minimal sensory deficit. TSP occurs primarily in the tropical and subtropical latitudes such as Jamaica, Columbia, South Africa, and south India.

To study the possibility of the occurrence of HIV and HTLV-I antibody in the United States before 1981, we examined sera obtained from pregnant women enrolled in the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke during the period 1959–1964. Included in these studies were samples from intravenous drug users, women with multiple pregnancies, women with abnormal children, and normal pregnant women. In addition, to indicate sensitivity of the serological tests used, we tested specimens from several current patient groups: homosexual patients with AIDS, homosexual controls without disease, patients with TSP, and controls.

MATERIALS AND METHODS

Patient Population

The pregnant women included in this study were enrolled in the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke, NIH. The study population has been well characterized [8,9]. Considerable data are available for health history, socioeconomic level, and ethnic grouping. Serum samples from over 55,000 pregnant women were collected during 1959–1964. For this study, serum samples from a subgroup of 310 pregnant women were selected. All samples tested were obtained from women near term. One hundred patients were selected, without regard to disease status, to represent the major geographic areas of the United States. An additional 53 patients had a history of intravenous drug addiction, 35 patients had had ten or more pregnancies, 35 patients had had babies with erythroblastosis fetalis; 15 patients had advanced carcinoma of the uterus at delivery, 15 patients developed evidence of abnormal cervical epithelium during pregnancy, and 21 had had children who developed malignant neoplasm during the first four years of age. For the latter two groups, 36 matched controls with normal outcome were included.

The current patient groups studied included 25 patients with AIDS, 25 homosexual patients with HIV antibody but without clinical signs of disease, and 54 homosexuals without disease from Los Angeles, California [10,11] as well as 20 patients with tropical spastic paraparesis (TSP) from the Seychelles Islands and 16 controls, of whom seven had other neurological diseases and nine were normal.

TABLE 1
Antibody to HIV and HTLV-I, Pregnant Women, 1959-1964

Patient Population	No. Positive/No. Tested	No. Positive/No. Tested
	HIV	HTLV-I
100 random selection	0/100 ^a	NT ^b
53 intravenous drug users	0/53	0/53
35 had ten or more pregnancies	0/35	0/35
35 had babies with erythroblastosis fetalis	0/35	0/35
15 had advanced cervical carcinoma at delivery	0/15	0/15 ^c
15 developed abnormal cervical epithelium during pregnancy	0/15	0/15
15 matched normal controls	0/15	0/15
21 had children who developed malignancies	0/21	0/21
21 matched normal controls	0/21	0/21
	0/310	0/210

^aTwo had initial ELISA-positive reactions.

^bNot tested

^cOne had initial ELISA-positive reaction.

Serological Tests

The enzyme-linked immunosorbent assay (ELISA) test was used as the initial test to detect HIV and HTLV-I antibody [12,13]. All ELISA tests were performed using commercially available reagents. The HIV ELISA reagents were obtained from Organon Teknika Corp. (Oklahoma City, OK; manufactured by Bionetics Lab Products, Charleston, SC), or Electronucleonics (ENI) (Columbia, MD). The HTLV-I ELISA reagents were obtained from the Dupont Company (Billerica, MA; manufactured by Biotech Research Labs, Inc., Rockville, MD). The tests were performed according to the directions supplied by the manufacturer. The appropriate positive and negative sera supplied by the manufacturer were used to verify the sensitivity of the tests. In addition, a panel of our own positive and negative control sera was included with each set of tests.

Indirect immunofluorescence assay (IFA) was also used for HIV and HTLV-I antibody determinations [13,14]. The reagents for the IFA were obtained from ENI. The slides for HIV used the HTLV-IIIb virus in H9 cells and non-infected H9 cells for the control antigen. For HTLV-I, the slides contained HTLV-I in HUT 102 cells and non-infected H9 cells for the controls. The tests were performed as recommended by ENI and appropriate positive and negative sera were included.

The Western blot tests were performed by ENI [14].

RESULTS

The results obtained from testing the serum samples from the pregnant women in the Collaborative Perinatal Project for HIV and HTLV-I are presented in Table 1. Only two of the 310 serum samples had ELISA HIV antibody reactivity. The medical history of these two women was reviewed. Patient 1 had a normal pregnancy with no significant clinical disease. The child born to this woman developed erythroblastosis

TABLE 2
Follow-Up Testing of HIV ELISA-Positive Sera from the Collaborative Perinatal Project

Patient	Organon ELISA ^a	ENI ELISA ^b	IFA HIV ^c	IFA H9 ^d	WB ^e
1	+	+	+	+	-
2	-	+	-	-	-

^aOrganon Teknika Corp., Oklahoma City, OK

^bElectronucleonics, Columbia, MD

^cIFA test using HTLV-IIIb infected cells

^dIFA test using control H9 cells

^eWestern blot

fetalis. A prior pregnancy had been uneventful, and a normal child had been born. Patient 2 had a normal pregnancy with no significant clinical diseases. The child born to patient 2 was normal. Patient 2 had had 11 previous pregnancies, two of which terminated in spontaneous abortions. The nine other children were healthy.

The results of retesting these two samples with the Organon and ENI ELISA reagents as well as the IFA and Western blot studies are presented in Table 2. Serum from patient 1 gave a positive test reaction in both of the ELISA methods and reacted in the IFA test with both the HTLV-III virus-infected cells and the H9 control cells; it was negative in the Western blot test. Therefore it was concluded that this sample was negative and that the reaction observed in the two ELISA tests and the IFA were nonspecific. The serum from patient 2 reacted only in the ENI ELISA kit; it was negative with the Organon ELISA reagents and the IFA test, and the Western blot test was also negative. Thus, none of the sera collecting during 1959–1964 were HIV antibody-positive.

Only one of the 210 serum samples from the pregnant women was positive with the initial HTLV-I ELISA reagents. This sample was from a patient with advanced carcinoma of the uterus. On subsequent ELISA tests, it was consistently negative. When tested on the HTLV-I IFA antigen slide and the H9 control cell slide, it was also negative. Thus, none of the sera collected between 1959–1964 were positive for HTLV-I antibody.

The results of testing AIDS patients, homosexual controls, TSP patients, and controls are presented in Table 3. Sera obtained from homosexuals with clinical signs of AIDS and abnormal T4/T8 ratios were all positive for HIV antibody with both Organon and ENI HTLV-III ELISA reagents; they were also positive for HIV antibody with the IFA test and negative with the H9 cell control. Sera from 25 homosexuals had HIV antibody by both of the ELISA tests and the IFA test but no significant clinical signs of disease; some of them had reversals of T4/T8 ratios. Homosexuals without clinical signs of disease or abnormal T4/T8 ratios had no HIV antibody by either of the HIV ELISA methods; the IFA tests were also negative. Sera from the TSP patients and controls were negative for HIV antibody with both the ELISA methods and the IFA tests. Seventeen of the 20 TSP patients were positive for HTLV-I antibody by both the ELISA and IFA test, as were sera from two of the seven patients with other neurological diseases. These two patients were diagnosed as having transverse myelopathy and multiple sclerosis. Sera from the nine normal control patients were negative for HTLV-I antibody. None of the sera from the AIDS patients or homosexual controls were positive for HTLV-I.

TABLE 3
Antibody to HIV and HTLV-I Patients with AIDS, Normal Homosexuals,
Patients with Tropical Spastic Paraparesis, and Controls

Patient Population	HIV	HTLV-I
25 AIDS patients	25/25	0/25
25 HIV-infected patients	25/25	0/25
54 Normal homosexuals	0/54	0/54
20 Tropical spastic paraparesis	0/20	17/20
7 Other neurological disease patients		
Syphilitis dementia	0/3	0/3
Peripheral neuropathy	0/1	0/1
Transverse myelopathy	0/1	1/1
Guillain-Barré syndrome	0/1	0/1
Multiple sclerosis	0/1	1/1
9 Normal controls	0/9	0/9

DISCUSSION

The ELISA test for HIV antibody has been licensed for use by blood banks as a method of screening and eliminating transmission of AIDS by blood and blood products [15]. Currently, at least seven manufacturers produce kits that are used not only in blood banks but also in seroepidemiological studies and in determining the antibody status of individuals. A number of initial false positives are expected; therefore, initial ELISA-positive tests are repeated in duplicate and, if the repeated tests are positive, they are confirmed by Western blot or IFA [12,13,14]. In low-risk populations such as normal blood donor populations, about 0.85 percent of the samples are positive on the initial ELISA test and about 0.28 percent are positive on repeat testing.

In our study we found two HIV ELISA antibody-positive samples from pregnant women during the time period 1959–1964 which were repeatedly positive by at least one of the tests. One of these samples was positive in IFA with both the HTLV-III infected and H9 control cells, and the other was IFA-negative. Both were Western blot-negative. Thus, they were negative for specific HIV antibody. In our study on these stored sera, about 0.58 percent of the samples were repeat ELISA-positive. This result is similar to samples expected in low-risk populations. The cause of these repeat ELISA-positive samples is not known. In one of our samples, it seemed that the false reading was related to the tissue culture cell antigen in which the virus was grown [14,16]. The cause of the second false ELISA reaction is not clear. Thus, in our study of a defined population, including drug users, we did not find confirmed evidence for HIV infection in the United States early in the 1960s.

Our data from a homosexual population is consistent with previous studies [2,10,11]. Homosexuals with clinical AIDS have HIV infection. Those homosexuals without evidence of clinical disease may or may not have HIV antibody. HTLV-I antibody was not detected in the homosexual population studied and is not associated with development of clinical AIDS.

In our study, we found on the initial test one HTLV-I ELISA-positive sample from the serum of a pregnant woman who had advanced carcinoma of the uterus at delivery. This sample was not positive on the repeat ELISA or IFA tests. Thus, we did not find

evidence of confirmed HTLV-I antibody in sera collected between 1959–1964. Our data indicate that HTLV-I is associated with TSP.

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