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Epidemiology of HIV-2 Infection in Spain

V. Soriano[1]*, M. Gutiérrez[1], E. Caballero[2], G. Cilla[3], J.L. Fernández[4], A. Aguilera[5], C. Tuset[6], F. Dronda[7], A.M. Martín[8], E. Carballo[5], I. López[9], J. González-Lahoz[1], the HIV-2 Spanish Study Group[10]

Human immunodeficiency virus type 2 (HIV-2) infection is endemic in West Africa, where it is responsible for many cases of AIDS. HIV-2-infected subjects have been described in other countries, mainly African immigrants, although infection in native individuals has been reported as well. The first cases of HIV-2 infection in Spain were identified in 1988. Through December 1995, 56 HIV-2-infected individuals have been diagnosed, primarily in large urban areas (23 cases in Madrid and 18 in Barcelona). All are African immigrants, except for 12 natives (21.4%), six of whom acquired the infection in endemic areas; the remaining six (2 women with numerous sexual partners and 4 homo/bisexual men) acquired the infection in Spain. Heterosexual transmission was probable in all but seven cases: five homo/bisexual males, a subject who likely acquired infection through parenteral exposure, and a child born to an HIV-2-infected mother. Nine patients (all Spanish born) have developed AIDS (16%), six of whom have died. In conclusion, HIV-2 infection is present in Spain at a low rate, and there is little evidence supporting an emerging ongoing transmission outside the population of African immigrants.

[1] Service of Infectious Diseases, CIC, Instituto de Salud Carlos III, C/Sinesio Delgado 10, 28029 Madrid, Spain.
[2] Service of Microbiology, Hospital Vall d'Hebrón, Barcelona, Spain.
[3] Service of Microbiology, Hospital Virgen de Aranzazu, San Sebastián, Spain.
[4] Service of Tropical Medicine, Consorci Hospitalari, Mataró, Spain.
[5] Services of Infectious Diseases and Microbiology, Hospital Xeral, Santiago, Spain.
[6] Service of Immunology, Hospital General Universitario, Valencia, Spain.
[7] Service of Microbiology, Hospital Penitenciario, Madrid, Spain.
[8] Service of Microbiology, Hospital Insular, Las Palmas, Spain.
[9] Hospital de Meixoeiro, Vigo, Spain.
[10] R. Ortiz de Lejarazu, J.M. Eirós, Hospital Clínico, Valladolid; J. Tor, R. Muga, Hospital Germans Trias i Pujol, Badalona; E. Pujol, Hospital Juan Ramón Jiménez, Huelva; E. Calderón, M. Leal, Hospital Virgen del Rocío, Sevilla; R. Martínez-Zapico, F. Pozo, Hospital La Paz, Madrid; J. del Romero, C. Rodríguez, Centro Sandoval, Madrid; F. Ulloa, Hospital Municipal, Vigo; E. Pérez-Trallero, Hospital Virgen de Aránzazu, San Sebastián; R. Bravo, A. Mas, G. García-Lerma, M.L. Pérez-Labad, M. Gómez-Cano, N. Villalba, Laboratory of Molecular Virology, Instituto de Salud Carlos III, Madrid; Spain. A. Heredia, A. Vallejo, I. Hewlett, Division of Transfusion Medicine, Food and Drug Administration, Bethesda, Maryland, USA.

The human immunodeficiency virus type 2 (HIV-2) was isolated in 1986 from two African patients with AIDS who did not present serological reactivity against HIV-1, the classical agent of the disease (1). Since then, more than 1000 HIV-2-infected subjects have been described in Europe. Almost all have been African immigrants or, less frequently, Europeans who lived in West Africa at some time in their lives or who had sexual contacts with people from that region (2). In Portugal, however, the native population constitutes the greatest proportion of cases of HIV-2 infection, and this retrovirus is responsible for 8% of the total number of AIDS cases. The relationship between this country and its historical colonies in West Africa could explain this circumstance (3). In Spain, the first three cases of HIV-2 infection were identified in 1988, occurring in three African immigrants living in Barcelona (4). These three and an additional 53 HIV-2-infected persons reported subsequently are the subject of this descriptive analysis.

Patients and Methods

Registry of Cases. In 1990 the HIV-2 Spanish Study Group was formed. One of its main objectives was the creation of a

national registry of cases of HIV-2 infection. Periodically, the data of that registry have been reported, and specific recommendations regarding epidemiological aspects and the most useful criteria for diagnosis have been issued. Studies of prevalence have been conducted by screening populations known to have an increased risk of infection. The data through 31 December 1995 have been analyzed in the coordinating center (Instituto de Salud Carlos III, Madrid).

Diagnostic Tests. The diagnosis of HIV-2 infection was made in all cases through detection of specific antibodies and, when cell samples were available, through further investigation of specific gene sequences by the polymerase chain reaction (PCR). The serological tests used were specific EIAs prepared with antigen obtained from supernatant viral lysates (ELAVIA-2, Diagnostics Pasteur, France) or synthetic peptides (HIV-2 EIA, Clonatec Diagnostics, France), line immunoassays designed with synthetic peptides (Pepti-LAV, Diagnostics Pasteur) or recombinant proteins (RIBA HIV 1+2, Ortho Diagnostics Systems, USA), and Western blot (New LAV-BLOT II, Diagnostics Pasteur).

Studies conducted since 1991 have employed several different EIAs designed for the detection of antibodies to both HIV-1 and HIV-2 (Abbott, USA; BioKit, Spain; Ortho, USA; Murex, UK; Behring, Germany; Pasteur, France). The analysis of specific serological tests for HIV-2 was reserved for those cases in which the Western blot yielded indeterminate results for HIV-1. In a study carried out in 1991 (5), more than 1000 HIV 1 and 2 EIA-seroreactive samples were analyzed in triplicate with a line immunoassay (Pepti-LAV) and Western blot for HIV-1 and HIV-2 (New LAV-BLOT I and II). Finally, since 1993 some laboratories have confirmed EIA-reactive samples using a Western blot that has incorporated together with the proteins of HIV-1, a specific HIV-2 peptide fixed on the bottom of the nitrocellulose strip (HIV BioBlot plus; BioKit, Spain). Samples that show reactivity to the HIV-2 peptide are further confirmed using a Western blot specific for HIV-2. Seropositivity for HIV-2 was established in all cases by following the WHO Western blot interpretation criteria (6) and confirming reactivity by another serological test, usually the Pepti-LAV.

The genetic diagnosis was performed using DNA extracted from patients' peripheral blood mononuclear cells. The previously described multitarget PCR method (7) was used most often, although different sets of primers and amplification procedures were also employed. In all the cases the specificity of the amplified product was confirmed with ^{32}P-labeled probes and further liquid hybridization, followed by electrophoresis in a polyacrylamide gel and autorradiography.

Results

Through December 1995, 56 cases of HIV-2 infection have been reported in Spain. The chronological analysis shows that the spread of HIV-2 in Spain is slow: 3 cases reported in 1988, 10 in 1990, 16 in 1991, 8 in 1992, 11 in 1993, 2 in 1994, and 6 in 1995.

Figure 1 shows the geographic distribution of the cases of HIV-2 infection. The majority (73.2%) were identified around the country's two largest urban areas: Madrid (n = 23) and Barcelona (n = 18). Although most of the individuals infected were African immigrants, 12 (21.4%) were native Spaniards, six of whom had apparently acquired the infection in endemic regions; the remaining six (a prostitute from Barcelona, another woman from Burgos who reported having numerous sexual partners, and 4 homo/bisexual men from Guipúzcoa) had been infected in Spain. The first cases of HIV-2 infection in Spanish residents were described in Galicia in 1991, in two seamen who had traveled to the African coasts. Heterosexual transmission was implied in all but seven cases (5 homo/bisexual males, a person who likely acquired infection accidentally through parenteral exposure, and a child infected by vertical transmission). All of the subjects denied parenteral drug use and had no history of blood transfusion.

Most of HIV-2 positive patients were males (50/56; 89.3%). Many of them had had multiple sexual partners, and more than half from whom information was available admitted to having had a venereal disease (22/43; 51%). With respect to the six HIV-2-infected women, three were native Spaniards and the others were immigrants (2 Africans living in Madrid and 1 Portugese living in Lleida). One of the Spanish natives is a prostitute living in Barcelona, who admitted to having frequent sexual contact with Africans. She denied use of intravenous drugs, transfusions, and travel to endemic areas. Another Spanish woman admitted to having had numerous sexual partners in Spain; she is coinfected with HIV-1 and HIV-2. The remaining Spanish woman is from Galicia and was the sexual partner of a male, also born in Spain, who died of AIDS caused by HIV-2 in 1993. The two African immigrant women are both asymptomatic at present. One is a prostitute, native to the Cape Verde Islands and the mother of the unique HIV-2-infected child mentioned previously. The other is a native of Ghana and is coinfected by HIV-1 and HIV-2 (8).

Four (7.3%) HIV-2-infected subjects were coinfected with HIV-1. In addition, a 23-year-old male, born in Mali and residing in Madrid, is seropositive for HIV-1, HIV-2, and HTLV-I. Although he has a low CD4+ lymphocyte count, he remains asymptomatic (9). Another subject, a 33-year-old male from Cameroon now residing in Madrid, is coinfected by HIV-2 and HTLV-II. He reported having had several sexually transmitted diseases (9). HTLV-I/II infection was excluded in

other 38 subjects with HIV-2 infection; the remaining could be not tested.

The prevalence of HIV-2 infection in African immigrants living in different Spanish geographical areas is variable, although it fluctuates between 1 and 3% in Madrid and Barcelona, respectively (10, 11). The population from West African countries has higher rates of infection, although in Spain some HIV-2-positive subjects have been identified as natives of other sub-Saharan regions, such as the Central African Republic and the Republic of South Africa.

Molecular studies were performed in 11 patients for the purpose of confirming HIV-2 infection. In all cases but one, the positive serological tests for HIV-2 corresponded to demonstration of specific gene sequences of the virus through PCR. In another three patients coexistence of gene sequences from both HIV-1 and HIV-2 was demonstrated.

To date, nine patients (all Spanish natives) have developed AIDS (16%), six of whom have died. The average duration of survival from the diagnosis of AIDS in these patients is five months, significantly lower than the more than one year survival described in Spanish patients with AIDS caused by HIV-1. In four patients knowledge of HIV-2 infection was available at the time they were admitted to hospital because of opportunistic diseases that had rapidly fatal outcomes. The six deceased patients had developed wasting syndrome, and one of them, a bisexual male, presented Kaposi's sarcoma. The average age of these AIDS patients with HIV-2 infection was 59 years, higher than the average for Spanish AIDS patients infected with HIV-1 (32 years). At least ten additional patients have a CD4+ lymphocyte count of $< 200/mm^3$; one of these is the sole pediatric patient, the six year-old son of an HIV-2-seropositive prostitute living in Madrid.

Discussion

Reported cases of HIV-2 infection worldwide surpass 3,000, most of which are in West Africa (2, 3, 12–14). Up to January 1990, 847 cases of HIV-2 infection had been reported in Europe (2). Although the majority were African immigrants living in Western Europe, HIV-2 infection has been reported amongst European citizens from numerous groups at risk of infection, including homosexuals in Portugal, Belgium, England, and France (2, 15, 16); drug addicts in Italy and Portugal (2); blood transfusion recipients in France and Portugal (2, 17, 18); and heterosexual partners of seropositive subjects in England, Portugal, Switzerland, and Holland (2, 19, 20). France and Portugal are the two European countries with the largest number of cases (2). In Spain 12 HIV-2 infected native Spaniards, five from Galicia, four from Guipúzcoa, and three from the Canary Islands, Barcelona, and Madrid, have been identified. Nine of them developed AIDS, and six have died.

As in other countries (2, 3, 12–15, 19, 20), most of the subjects with HIV-2 infection identified in Spain have acquired the infection heterosexually, although cases of vertical, parenteral, and homosexual transmission exist. In the Spanish register males predominate (89.3%); only six HIV-2-infected women have been reported, a rate similar to that reported in other European countries and in the USA (2, 3, 14, 21). These findings, however, do not agree with those for Portugal and the African countries, where HIV-2 infection affects both sexes alike (2, 3, 14, 22), probably because in these areas the heterosexual circulation of HIV-2 has been occurring over many years (3, 23).

One subject with vertically acquired HIV-2 infection has been identified, the 6-year-old son of a prostitute native to Cape Verde Islands who resides in Madrid. He has a severe cellular immunodeficiency, although he remains AIDS free. The vertical transmission of HIV-2 is rare and can occur during pregnancy, at the time of delivery, or through breastfeeding (3, 14). However, the vertical route is less efficient for the transmission of HIV-2 compared to HIV-1, so that less than 2% of the children of HIV-2 seropositive mothers acquire the infection (14, 24–28). The lower viremia seen in HIV-2 carriers compared to that observed in HIV-1-infected persons could explain this lower rate of transmission (3, 14, 29, 30).

The first descriptions of AIDS in HIV-2-infected subjects appeared in 1986 (1). Clinical manifestations were similar to those observed in HIV-1-infected persons with AIDS (31); however, it was soon clear that most of the HIV-2-infected subjects were asymptomatic (2, 3). Unlike HIV-1, HIV-2 infection seems to be less frequently associated with disease. Similarly, in the Spanish series described here, only nine (16%) HIV-2-positive subjects developed AIDS. Currently, it is thought that the long latency period that exists between the moment of acute infection and the appearance of symptoms is due to a lower virulence of this virus

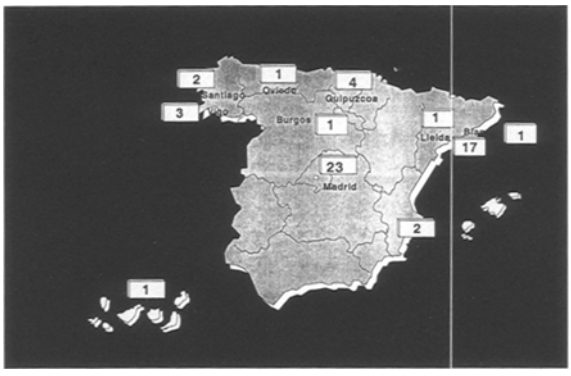

Figure 1: Geographic distribution of 56 cases of HIV-2 infection identified in Spain through December 1995.

(14, 17, 29, 31–33). It is estimated that the progression to group IV of the Center for Disease Control's classification (1987) is three to four times faster in HIV-1-infected subjects than in HIV-2 carriers (32). Finally, the survival period after diagnosis of AIDS is also longer for HIV-2 infected patients than for those infected with HIV-1 (3, 30). The short survival period of the six Spanish patients with AIDS due to HIV-2 who have died can be explained by the lack of medical care until very advanced stages of the disease; most of the patients did not have previous knowledge of their retroviral infection.

One of the patients was diagnosed with Kaposi's sarcoma. He was from the Canary Islands and had been engaged in homosexual relations with multiple partners. Kaposi's sarcoma has already been described in HIV-2-infected patients, although very infrequently (29).

The identification of HIV-2-infected Spanish natives who have never visited endemic areas raises caution against underestimating the spread of HIV-2 and justifies continuous seroepidemiological surveillance (34). In Spain, at least five subjects (1 prostitute and 4 homo/bisexual men) most likely acquired HIV-2 infection within the country. However, it is unlikely that the spread of HIV-2 could reach the rate of HIV-1 spread, due to its lower transmissibility (3, 14, 29, 33).

Thus far, no blood donors infected by HIV-2 have been identified in Spain. In September 1993 screening for HIV-2 antibody became mandatory in Spanish blood banks. No cases of HIV-2 infection were recognized in the first 100,000 donors. In a study of 100,114 samples of blood donors carried out in France in 1987, none was positive for HIV-2 (35). Likewise, no HIV-2-infected donations were found in 22,694 samples in the USA in 1987 (36) or in later studies that have included more than 26 million blood donations (21, 37, 38). However, one French donor who had escaped detection by the oldest serological tests, designed to recognize antibodies to HIV-1, has recently been identified as being HIV-2 positive (18). Moreover, the first two cases of HIV-2 infection have recently been detected in blood donors in the USA (39).

The results of this study show that HIV-2 infection is infrequent in Spain, although there is evidence of an increasing spread of the virus outside the African immigrant population. An effective strategy in order to monitor the spread of HIV-2 infection might consist of analyzing those subjects who show reactivity on combined HIV 1+2 EIAs but who remain indeterminate on the HIV-1 Western blot. Using this strategy, at least 11 HIV-2-infected subjects have been identified in the USA (21, 36–40). In Madrid the first cases of HIV-2 infection were identified in 1991 following this approach (41), and other cases have now been recognized similarly (42).

Given the antigenic similarity between HIV-1 and HIV-2, the diagnosis of HIV-2 infection can be difficult using serological tests alone (38). Thus, molecular biology techniques that make it possible to analyze the presence of specific retroviral gene sequences are often necessary in order to confirm HIV-2 infections and to distinguish between HIV-1 and HIV-2 coinfections and serological cross-reactivity (43, 44). However, it should be noted that the sensitivity of PCR may be lower for HIV-2 than for HIV-1. In fact, false-negative PCR results are not infrequent in asymptomatic HIV-2-infected persons. Two main reasons explain this observation: first, fewer infected peripheral lymphocytes carry HIV-2 (7, 29, 43), and second, the different HIV-2 strains are highly genetically variable (7, 45). The performance of double or nested PCR (46) or the analysis of larger quantities of sample (high-input PCR) clearly compensate for this limitation.

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