Introduction of Human Immunodeficiency Virus Type 2 Infection in the Philippines

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The aim of this study was to describe and document the first case of human immunodeficiency virus type 2 (HIV-2) in the Philippines by using serological and molecular techniques and to compare the diversity of this strain to that of strains from other countries. With the introduction of HIV-2 into the country and the presence of diversified strains of HIV-1, the use of highly sensitive assays to detect all these strains is recommended.

CASE REPORT

The patient is a married 43-year-old female who was previously tested and was found to be reactive to a human immunodeficiency virus (HIV) screening test; she was referred to our laboratory for confirmatory testing in September 2000. She has been working as an overseas contract worker for the past several years, and she worked last in Malaysia. She admitted to having had several sexual relationships with foreigners, the most recent being contact with an Indian (1998) and an American (1999) national. The patient was still asymptomatic at the time of testing, with an absolute CD4 lymphocyte count (Coulter EPICS XL) of 427 cells/µl.

An anti-HIV antibody assay using Serodia HIV 1/2 (Fujirebio, Tokyo, Japan) gave a nonreactive result to HIV type 1 (HIV-1)-sensitized particles and a reactive result to HIV-2-sensitized particles. Indirect fluorescent-antibody testing showed negative fluorescence to HIV-1 LAI-infected T lymphocytes and positive fluorescence to HIV-2 GH1-infected T lymphocytes. Confirmation was done with an HIV-2 Western blot (New Lav Blot II; Bio-Rad), and eight bands were detected, namely, bands corresponding to p16, p26, p34, and p56 of Gag, p68 of Pol, and gp36, gp105, and gp140 of Env. Nested PCR was then performed to further confirm that the case is HIV-2 and not just a nonspecific reaction. Proviral DNA from peripheral blood mononuclear cells was amplified by PCR by using primers selected from previously described HIV-2 long terminal repeat (LTR) primers (outer primers JA1-163 and inner primers OG01 and G24) (3, 4). Product was visualized by agarose electrophoresis coupled with ethidium bromide staining. DNA extracted from CEM-uninfected and GH1-infected cells was included as a negative and positive control, respectively. Proviral DNA testing with these specific HIV-2 LTR primers gave a positive result. Sequencing was also done to confirm that this is a true HIV-2 infection and also to compare the sequence of our local strain to previously published sequences of other strains isolated from other countries, including those of three Indian strains from Mumbai that we had isolated. The cell lysate was subjected to amplification by using the outer primers JA-163 (nucleotides [nt] 6827 to 6851) and JA-166 (nt 7316 to 7340) and inner primers JA164F (nt 6911 to 6935) and JA165B (nt 7277 to 7301) (1), which are specific for regions flanking the V3 domain of HIV-2. Amplified product was sequenced by using Big Dye Terminator Cycle sequencing ready reaction mixture (Applied Biosystems) containing 0.5 µM primer JA164F. Sequencing and data collection was done by using an ABI 310 genetic analyzer. Multiple alignment of nucleotide sequence and phylogenetic analysis was done by using CLUSTAL W, version 1.7. Based on sequence alignment (Fig. 1A), our strain (A_2Ph01) clustered phylogenetically within HIV-2 subtype A (Fig. 1B). This is the first documented case of HIV-2 in the Philippines, and although we cannot absolutely conclude whether the source of infection was from within or outside of the country, there is greater feasibility that the patient acquired the infection from sexual contact with foreigners.

HIV-2 is closely related to HIV-1 and was first reported to be associated with AIDS in 1986. HIV-2 infection was first detected in, and is mostly confined to, West African countries. However, HIV-2-infected individuals have also been identified in other continents. In Europe, Portugal and France reported the highest number of cases among homosexual men, intravenous drug users, transfusion recipients and hemophiliac men (7). In the Western hemisphere, rare cases of HIV-2 infection have also been reported from Brazil, Canada, and the United States. In Asia, 95% of the reported HIV-2 cases were from India (5).

After more than a decade since the first reported case of HIV in the Philippines, in 1984, surveys conducted by the National HIV Surveillance Program among high-risk groups (sex workers and intravenous drug users) of the population show a low and slow HIV transmission, with a prevalence rate of 0.1% (1993) to 1% (2000). As of July 2002, the HIV AIDS
registry of the Department of Health has confirmed a total of 1,733 HIV antibody-positive cases (6). Thirty percent of cases were among overseas Filipino workers, of whom most (38%) were seafarers. The most common mode of transmission is still through heterosexual contact.

Serologically, most of these reported cases are reactive to HIV-1, and some are reactive to both HIV-1 and HIV-2. But some reports indicate that serologic techniques may overestimate the presence of HIV-2 or HIV-1 and -2 dual infections due to cross-reactivity of antibodies to antigens of both types, infection by one virus and exposure to a second one, or infection with a putative intermediate virus (5).

Several studies conducted on HIV patients in the Philippines have proven the presence of diverse multiple genetic group M subtypes of HIV-1. Based on sequencing of the env C2-V3 segment of gp120, five HIV-1 subtypes (A, B, C, D, and E) were identified, with subtype B being predominant, followed by subtype E (8, 10). Succeeding studies showed the presence of circulating recombinant forms (A/B, A/E, A/F, and A/G) (2). The high genetic variation of HIV-1 in the country and the subsequent introduction of HIV-2 have important implications for the sensitivity and specificity of the diagnostic tests currently used. Early diagnostic and viral load monitoring assay have been developed initially only for subtype B viruses, hence resulting in false-negative results for screening or indeterminate results for Western blot tests or an inability of the kit to detect or correctly quantify viral RNA in plasma from patients infected with non-subtype B strains of HIV-1 as well as HIV-2 (9). Although most of the commercial kits currently available are capable of detecting most forms of HIV-1 and its identified subtypes as well as those of HIV-2, there is no assay available as yet to detect and quantitate HIV-1 group O and

FIG. 1. Comparison of our local strain (A_2Ph01) with other HIV-1 and HIV-2 isolates. (A) HIV-2 sequence alignment. A multiple sequence alignment of HIV-2 strains based on envelope V3 region is shown. The alignment shows a comparison of our local strain (A_2Ph01) and HIV-2 subtype A (8) and subtype B (3) strains from the database, including A_ISY (Gambia), A_ST (Senegal), A_D1024 (India), A_CAM2 (Guinea-Bissau), A_ROD (Cape Verde Islands), A_BEN (Mali), A_GH1 (Ghana), A_885 (West Africa), A_UC1 (Ivory Coast), B_D205 (Ghana), B_EHOA (Ivory Coast), and three unpublished Indian isolates from private clinics, A_JKM 280, A_JKM 281, and A_JKM 285. Dots denotes nucleotides similar to those of the local isolate (A_2Ph01). (B) Phylogenetic relationship between our isolate (A_2Ph01) and other published isolates (HIV-1 and HIV-2), showing their genetic relationship.
HIV-2 strains. Therefore, continued surveillance and monitoring is necessary, since even slight changes in the antigenic structures of some HIV-1 variants may affect the sensitivity of existing diagnostic kits. The results of this study confirm the presence of HIV-2 infection in the Philippines. The use of the conventional serologic assays that detect HIV-1 and HIV-2 is therefore recommended for accurate diagnosis. This study further demonstrates that the high number of Filipinos traveling to and from the different regions of the world, specifically the overseas Filipino workers, plays an important role in the introduction of diverse HIV strains into the country. This study further suggests that overseas contract workers be included in the surveillance program and that an HIV-2 determination be included in all diagnostic tests.

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REFERENCES