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HIV-2 Infection in Thailand

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SUMMARY **Neither the seroprevalence of HIV-2 nor the sensitivity of enzyme immunoassays for the detection of antibodies to this retrovirus have been defined in Thailand. We, therefore, investigated these enigmas using banked sera previously screened for HIV-1 by a test that did not distinguish between HIV-1 and HIV-2. All 1,013 HIV-seroreactive specimens were positive to HIV-1 on retesting, and 740 (73%) were reactive to both HIV-1 and HIV-2. The thirty-six samples that reacted with HIV-2 at a titer of ≥ 1:4,096 were further tested to discriminate between HIV-1 and HIV-2 by immunoblot assays incorporating HIV-2 recombinant proteins. One specimen was untypeable, but all others were determined to be HIV-1. Seventy-three percent of sera from Thai HIV-1 infected subjects cross-reacted with HIV-2, but not a single case of HIV-2 infection could be confirmed. The finding suggests low prevalence of HIV-2 infection in Thailand and that current testing for HIV-2 antibody is not necessary in Thai population.**

The acquired immunodeficiency syndrome (AIDS) is caused by at least two human immunodeficiency viruses (HIV), type 1 and type 2. HIV-1 is prevalent worldwide, whereas HIV-2 appears to be largely confined to Western Africa.[1] In areas where both retroviruses occur, HIV-1 prevalence is increasing, but that of HIV-2 prevalence is not.[2] The incubation period for HIV-2 AIDS may be longer than that of HIV-1, although few cases have been studied.[3]

The HIV-2 genome exhibits approximately 60% homology to the HIV-1 genome in the *gag* and *pol* regions and 30-40% homology in less conserved genes such as *env*.[4] Serological studies indicate that HIV-1 and HIV-2 share common core antigenic epitopes but that envelope glycoproteins are less cross-reactive.[5] The sensitivity of HIV-1 enzyme immunoassays (EIAs) for the detection of antibodies to HIV-2 has been reported to range from 30 to 90%.[6,7] A number of manufacturers have, therefore, increased the sensitivity for detecting HIV-2 antibody by combining HIV-1 and HIV-2 antigens. Two cases of HIV-2 infection have been identified in foreigners in Thailand,[8] but the seroprevalence of HIV-2 in the Thai population has not been documented. We, therefore, investigated the prevalence of HIV-2 infection and cross-reactivity between HIV-1 and HIV-2.

MATERIALS AND METHODS

Test samples

One thousand and thirteen HIV-reactive sera had been collected between January 1997 and November 1999 from four different areas of Thailand: Bangkok (664 specimens), Rayong (southeast of Bangkok; 81), Chonburi (southeast of Bangkok; 115) and Chiang Mai (northern part of Thailand; 153). These banked sera had been previously tested by commercial kits for the detection of both HIV-1 and HIV-2. Six hundred and sixty-four

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samples were tested first by Genelavia mixt (Sanofi Diagnostics Pasteur Ltd., France) and then by Serodia HIV (Fujirebio Inc., Tokyo, Japan). Eighty-one were reactive by Serodia HIV and also by Axsym HIV-1/HIV-2 (Abbott GmbH Diagnostika, Germany). One hundred and fifteen samples were reactive by Sero-dia HIV and Capillus HIV-1/HIV-2 (Cambridge Diagnostics Ireland Ltd., Ireland). One hundred and fifty-three samples were first tested by Vironostika HIV Uniform II (Organon Teknika BV, The Netherlands) and then by Serodia HIV.

Retesting for antibody to HIV-1 and HIV-2 (Fig. 1)

All sera were retested for antibody to HIV-1 and HIV-2 by Serodia HIV-1/2 (Fujirebio Inc., Japan), an agglutination assay using separately inactivated HIV-1 and HIV-2 virus lysate antigens coated onto gelatin particle carriers. Twenty five microliters of 1:8, 1:16, and 1:32 dilutions of each serum were tested against 25 µl of unsensitized, HIV-1 sensitized, and HIV-2 sensitized particles (final dilutions of 1:16, 1:32 and 1:64), respectively, on a U-type microplate. The plate was kept at room temperature for 2 hours, and the agglutination patterns were then compared with those of the reagent controls and the results interpreted according to the manufacturer's instructions. Specimens that reacted negatively with unsensitized particles but agglutinated with sensitized particles (final dilution 1:32 for HIV-1, 1:64 for HIV-2) were defined as HIV positive. HIV positive sera were then subjected to serial 2-fold dilutions from 1:32 to 1:1,024 and those with a clear agglutination pattern 2+ (agglutinated particles spread out uniformly covering the bottom of the well) against HIV-2 at a concentration > 1:512 were diluted further from 1:512 to 1:8,192. Specimens with HIV-2 reactivity were studied further[9] to discriminate between HIV-1 and HIV-2 infection.

Testing for HIV-2 infection

Immunoblotting was performed using an assay that incorportates both HIV-1 recombinant proteins (p17, p24, and p31) and synthetic peptides (gp41 and gp120) and HIV-2 envelope (gp36, gp105) synthetic peptides (Inno-Lia HIV confirmation assay, Innogenetics N.V., Zwijnaarde, Belgium). Antigens were coated as seven discrete lines on each nylon strip. Sera which gave a pattern that could not be differentiated into either HIV-1 or HIV-2 were classified as non-typeable and tested further by a strip immunoblot assay using recombinant proteins (RIBA HIV-1/HIV-2

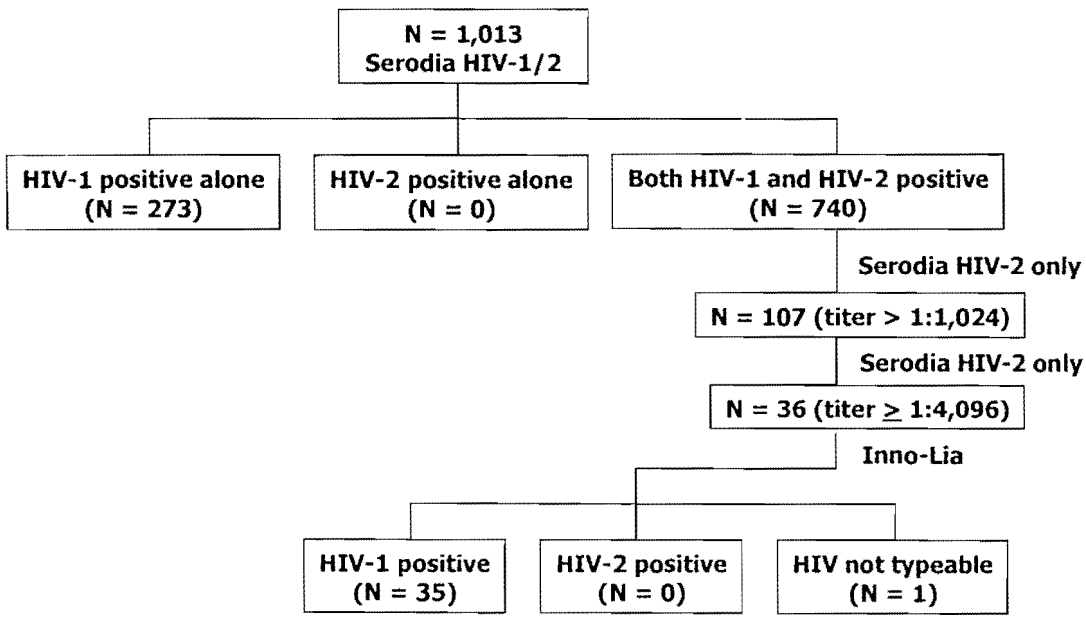

Fig. 1 Flowchart of retesting for antibodies to HIV-1 and HIV-2 in sera of individuals from different areas of Thailand.

SIA, Chiron Corp., CA, U.S.A.). This assay uses the HIV-1 recombinant proteins p24, p31, gp41, and gp120 and the HIV-2 p26 protein. A synthetic HIV-2 envelope peptide, gp36, is also present on each strip. Tests were performed and interpreted according to the manufacturer's instructions.

RESULTS

All 1,013 HIV seroreactive specimens were positive for anti-HIV antibody when retested by Serodia HIV-1/2. Seven hundred and forty (73%) sera were reactive to both HIV-1 and HIV-2. Only 273 sera (27%) were reactive only to HIV-1, and none were reactive only to HIV-2. One hundred and seven samples (15%) were strongly reactive, i.e. with HIV-2 antibody titer > 1:1,024 (Table 1), and 36 of those were reactive at a titer of ≥ 1:4,096. Thirty-five of these sera (titer ≥ 1:4,096) were confirmed to be anti-HIV-1 antibody positive. However, one was untypeable by Inno-Lia as either HIV-1 or HIV-2. Further testing using RIBA confirmed that the subject was HIV-1 infected (Table 2).

Table 1 HIV antibody titers against HIV-2 antigen from 740 sera that reacted with both HIV-1 and HIV-2

Reciprocal titer	Number of cases	%
64	104	14.0
128	80	10.8
256	117	15.8
512	91	12.3
1024	119	16.1
> 1,024*	122	16.5
> 1,024**	107	14.5
Total	740	100

* titer with agglutination pattern +/-, or 1+
** titer with agglutination pattern 2+

DISCUSSION

The 73% cross-reactivity rate that we observed between HIV-2 whole virus antigens and HIV-1 infected Thai sera is higher than the 13-30% rates reported from Japan and the 55% rate reported from Ghana.[10,11] There are several possible explanations for these discrepancies. The HIV-1 strains circulating in Thailand could induce more broadly-reacting antibody than strains from other areas, and it is also possible that antibody titers measured by particle agglutination were higher in our patient population.

No case of HIV-2 was identified among the 1,013 specimens tested. This finding is sup-

Table 2 Results of typing one serum sample that was untypeable by Inno-Lia HIV confirmation assay and RIBA HIV-1/2 SIA.

Inno-Lia HIV confirmation assay								RIBA HIV-1/2 SIA					
gp120	gp41	p31	p24	p17	gp105*	gp36*	**Result**	gp120	gp41	HIV-2 env peptide**	p31	p24/p26	**Result**
3+	3+	3+	3+	3+	-	2+	HIV	4+	4+	-	4+	4+	HIV-1

* HIV-2 specific protein

HIV-1
- one HIV-1 antigen (gp120 or 41) is positive (≥ 1+)
- both HIV-1 (gp120 and gp41) antigens are positive (≥ 1+)

HIV-2
- one HIV-2 antigen (gp105 or gp36) is positive (≥ 1+)
- both HIV-2 antigens (gp105 and gp36) are positive (≥ 1+)

HIV positive but not typeable
- both HIV-1 and HIV-2 specific antigen lines are reactive

** HIV-2 specific antigen

HIV-1
- gp41 and any other HIV-1 antigen band ≥ 1+, but HIV-2 env band < 1+

HIV-2
- HIV-2 env and any other HIV-1 band ≥ 1+, but gp120 band < 1+

HIV positive for HIV-1 and HIV-2
- HIV bands ≥ 1+, but the pattern does not meet the criteria for HIV-1 or HIV-2 positive

ported by data from HIV surveillance in Thai military conscripts. Approximately 277,947 young men were screened during 1995-1999 using two sequential enzyme immunoassays which incorporate HIV-1 and HIV-2 peptides or recombinant antigens. No case of HIV-2 was identified out of 5,125 HIV-1 reactive sera confirmed as HIV-1 by Western blot. Ten of these sera showed reactivity at the HIV-2 peptide indicator band, but all ten were determined to be HIV-1 by the more specific recombinant-based antigen assay (unpublished data).

Our study emphasizes that locally acquired data on cross-reactivity should be gathered where diagnostic kits are sold. Until HIV-2 is shown to be more of a threat to the public health of Thailand, it may not be necessary to use tests capable of detecting both HIV-1 and HIV-2.

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