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Co-Existence of HIV-1 Subtypes B′ and E Infections among Thai Injecting Drug Users

Suthon Vongsheree[1], Tipwan Phutiprawan[2], Pongnuwat Sri-ngam[1], Hansa Thaisri[1], Wiroj Puangtabtim[1] and Pathom Sawanpanyalert[1]

SUMMARY **Subtypes B′ and E are the two major subtypes of HIV-1 among injecting drug users (IDU) in Thailand. However, there are not many reports on subtype distribution during the early epidemic. Random blood specimens collected during 1994-2000 from 3,286 IDU at the Thanyarak Hospital were tested for HIV antibody and subtyped by using peptide binding enzyme immunoassay. The prevalence rate of HIV infection was 36.8%. All HIV-seropositive IDU were ascertained for "year of first HIV seropositivity" from their medical records. Of 1,512 HIV-seropositive samples, 1,408 (93.1%) were typeable. During 1987-1988, the proportion of subtype B′ was as high as 80% but decreased rapidly to 27.6% during 1999-2000. At the same time, the proportions of subtype E increased correspondingly (Chi-square test for the trend, $p < 0.05$). The relatively high proportion of subtype E among IDU since an early stage of the epidemic suggests early co-existence of both subtypes and needs further investigation.**

The first HIV case in Thailand was reported in 1984.[1] Injecting drug use has been recognized as a major risk factor for HIV infection since early in the epidemic. The prevalence rates of HIV infection among injecting drug users (IDU) in Bangkok were relatively high. They rose from 0-1% during 1985-1987 to 32-43% during 1988 and beyond.[2-5] At the same time, there were reports of strikingly high HIV prevalence rates among female commercial sex workers in northern Thailand of around 40%.[3,6] Sexual transmission was then recognized as another major risk factor for HIV infection. During 1992 to 1993, there were reports that the HIV-1 circulating in Thailand had two main envelope subtypes, i.e. B′ (Thai B) and E. It was believed that these two subtypes were introduced independently and segregated by different routes of transmission, i.e. B′ mainly through injecting drug use and E mainly through sexual transmission.[7-9] As high as 80-90% of HIV-1 among heterosexual risk groups were of the E subtype. On the other hand, subtype B′ was primarily found among IDU with the proportion of as high as 75-94% during 1988 to 1991. The proportion of subtype B′ among IDU decreased to 56-67% during 1994 and 1995. At the same time, subtype E was found with increasing frequency, especially among young IDU.[9-11] However, serial observations of HIV subtypes among IDU and other high-risk groups were not available in the past due to several reasons. One of the main reasons is that the methods to subtype HIV-1 such as the peptide binding enzyme immunoassay (PEIA) were only established in 1992.[12] Therefore, there is not much information on HIV-1 subtypes during the early epidemic. This study reports distribution of HIV-1 subtypes among IDU in a major substance abuse treatment facility in Thailand during 1987 to 2000.

From the [1]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, [2]Thanyarak Hospital, Department of Medical Services, Ministry of Public Health, Pathumthani, Thailand.
Correspondences: Suthon Vongsheree

MATERIALS AND METHODS

Setting

This study was the result of a collaboration between the Thanyarak Hospital of the Department of Medical Services and the National Institute of Health (Thai NIH) of the Department of the Medical Sciences, Ministry of Public Health of Thailand. The hospital is located about 30 kilometers north of Bangkok. It is the largest substance abuse treatment facility in Thailand. Each year, it treats approximately 11,000 drug users of which 1,700 are IDU. The hospital has developed HIV counseling and testing services for its patients since the beginning of the epidemic. As per hospital policy, all drug users seeking medical care at the hospital are routinely asked to undergo HIV counseling and testing, regardless of their previous HIV serostatus. The counseling and testing services are provided on a voluntary basis with written consent from the patients and in a confidential manner. All drug users treated in the hospital are asymptomatic or mildly symptomatic. Severely ill drug users are treated elsewhere. The HIV prevalence rates among IDU treated in the hospital were as follows: 37.2% in 1992, then the rates increased to a maximum of 45.3% in 1997 and remained stable at 42-43% during 1998-2000.

Clinical specimens

As a joint effort between the Thanyarak Hospital's laboratory and the HIV/AIDS laboratory of the Thai NIH to determine the HIV-1 subtype distribution among IDU seeking medical care at the hospital, the hospital agreed to send random samples of blood specimens of IDU in 1994, 1997, 1999, and 2000 to the HIV/AIDS laboratory. The specimens were obtained from every patient seeking medical care for a period of 6 consecutive months in each of the specified years. They were initially tested for HIV-1 serostatus (HIV-1/HIV-2 Third Generation, Abbott GmbH Diagnostika, Germany) and the urine samples were tested for concurrent heroin/opium use by using the opiate test EMIT® d.a.u. (Syva USA) at the hospital laboratory. The sera were later sent to the Thai NIH's HIV/AIDS laboratory for confirmation of HIV-1 serostatus and for HIV-1 subtyping. In addition, all blood specimens which tested HIV-1-positive at the hospital laboratory in 1996 were also sent for HIV-1 subtyping at the Thai NIH's HIV/AIDS laboratory. Due to limited resources, specimens from the years 1995 and 1998 were not sent. Together with the blood specimens, the following information was available for analysis, i.e. current age, gender, result of testing for urinary opiates, year of first HIV seropositivity (ascertained from the patients' medical records), and age at first HIV seropositivity (ascertained from the patients' medical records).

PEIA for HIV subtyping

HIV-1 subtyping was done at the Thai NIH's HIV/AIDS laboratory by using PEIA, as described by Pau.[12] This test has been validated with the standard methods, i.e. Heteroduplex Mobility Assay (HMA) and DNA sequencing. In brief, peptide E and B′ (kindly provided by the HIV/AIDS Collaboration, Thailand) were solubilized in 0.1 M carbonate buffer, pH 9.6 to a final concentration of 5 μg/ml. An 8 x 12 microtiter plate (Immulon II, Dynatech Laboratories Inc., USA) was coated with 100 μl/well of the peptide solution at 4°C overnight. Then, the plate was washed twice with PBS-T containing 0.01 M phosphate buffered saline, pH 7.2 and 0.05% v/v Tween-20 (Sigma Chemical Co., USA) and thereafter the plate was blocked with 5% skim milk (E Merck, Darmstadt, Germany) in PBS-T at 4°C overnight. The coated plate was washed 5 times with PBS-T, vacuum sealed and stored at -70°C until use. The performance of the analytical steps was optimized by block titration of the control B′ and E sera (kindly provided by the HIV/AIDS Collaboration, Thailand) as well as the conjugate (rabbit anti-human IgG-HRP, Dako A/S, Glostrup, Denmark). Diluted sera (100 μl of 1/200-1/500) in milk buffer were added to the well and incubated for 1 hour at 37°C. Unbound antibodies were removed by washing 5 times with PBS-T. The bound complexes were detected by adding 100 μl of BM-blue (Roche Diagnostics GmbH, Mannheim, Germany) into each well and observing at room temperature for 10 minutes. The reaction was then stopped by adding 100 μl of 1 N H_2SO_4 into each well. Absorbance was read at 450/630 nm and a cut-off value of 0.3, which was approximately equal to the mean absorbance of the negative control plus 7 SD, was used to identify positive EIA. For typeable cases, the absorbance of the specific peptide should be more than 3 times that of the others. For the dually reactive ones, the sera were further subtyped by antigen limiting EIA as described by Gaywee.[12]

Statistical analysis

Proportions of HIV subtypes and their associated 95% confidence intervals (95% CI) were calculated. The proportions across various groups and categories were

compared by using Chi square test or Chi square test for trend, as indicated. Mean, median and standard deviation were calculated for continuous variables with an approximately normal distribution. A *p*-value of 0.05 was taken to denote statistical significance when the categorical and the continuous variables were tested with Chi-square or t test, respectively.

RESULTS

There were blood specimens from 1,146, 988, 424, and 250 IDU in 1994, 1997, 1999, and 2000, respectively. The HIV-1 prevalence rates among IDU seeking medical care at the Thanyarak Hospital in 1994, 1997, 1999 and 2000 were 32.9% (377/1146), 40.7% (403/988), 37.3% (158/424) and 38% (96/250), respectively. Characteristics of these IDU are shown in Table 1.

The overall HIV prevalence rate for the four years was 1,034 out of 2,808 or 36.8% (95% CI = 36.6%-37.6%). Out of the 2,808 IDU, results of urinary opiates were available for 1,662 IDU. Out of the 1,662 IDU, 1,372 (82.6%) were tested positive for urinary opiate indicating active or current use of heroin or opium. HIV-positive IDU had a higher positive urinary opiate rate (593/1372 or 43.2%) than that of HIV-negative IDU (64/290 or 22.1%) ($p < 0.05$).

The mean age of HIV-positive IDU (mean = 26.7 median = 25.9, standard deviation = 6.9) is higher than that of HIV-negative IDU (mean = 25.4 years, median = 23.7, standard deviation [SD] = 7.4) ($p < 0.05$ by t test). The overall mean age of the IDU is 25.9 Years (median = 25.2, standard deviation = 7.6). The male-to-female sex ratio of HIV-positive IDU is 24:1 and similar to that of HIV-negative IDU (21:1).

HIV subtypes

Of the 1,408 HIV-positive IDU, 502 (35.6%) were of subtype B'. The characteristics of IDU with subtypes B' and E are shown in Table 2. The median age of the HIV-positive IDU, i.e. 26.0 years, is used to compare the HIV-1-infected IDU with the two different subtypes. The IDU infected with the two subtypes do not differ significantly in terms of gender or urinary detection of opiates. However, those infected with subtype B' tend to be older and possibly infected earlier (as reflected by the year of first HIV seropositivity) than those with subtype E.

HIV subtypes and gender

Among the 971 HIV-positive IDU, 933 (96.1%) are male. The proportion of HIV subtype B' among male IDU is 37.1% (346/933), as compared to 34.2% (13/38) among female IDU ($p > 0.05$).

HIV subtypes and year of first HIV seropositivity

There were 1,191 HIV-positive cases whose year of first HIV seropositivity could be ascertained from their medical records. The year of first HIV seropositivity ascertained from the HIV-positive IDU went back as far as 1987.

Table 1. Characteristics of injecting drug users at the Thanyarak Hospital in this study (N = 3,286)

	1994	1996	1997	1999	2000	Total
Sample size	1,146	478*	988	424	250	3,286
No. (%) of HIV-1 positive IDU	377 (32.9)	478*	403 (40.7)	158 (37.3)	96 (38)	1,512 (36.8)
No. (%) of subtype B'	176 (46.7)	142 (29.7)	115 (28.5)	48 (30.4)	21 (21.9)	502 (33.2)
No. (%) of subtype E	169 (44.8)	294 (61.5)	265 (65.8)	108 (68.4)	70 (72.9)	906 (59.9)
No. (%) of the non-typeable	32 (8.5)	42 (8.80	23 (5.7)	2 (1.2)	5 (5.2)	104 (6.9)
Ratio of subtypes B':E	49/51	32.6/67.4	30.3/69.7	30.8/69.2	23.1/76.9	64.3/35.7
Opiate test						
No. positive (%)	ND	ND	960 (97.2%)	361(85.1)	51(20.4)	1372(82.6)
Gender						
Male/female (ratio)	1,108/38 (29:1)	ND	963/25 (38:1)	421/3 (140:1)	96/0	1,846/39 (47:1)
Age range	16-63	16-58	16-53	15-54	19-50	16-63
Median age-years (SD)	26.0 (7.9)	ND	24.2 (7.4)	25.5 (7.3)	27.9 (6.9)	26.7 (7.6)

Note: *All were anti-HIV-1 positive cases. ND, no data available; SD, standard deviation

Table 2. Characteristics of IDU with HIV-1 subtypes B′ and E (N = 1,408)

	Subtype B′ No. (%)	Subtype E No. (%)	Total No.
Total	502 (35.6)	906 (64.4)	1,408
Male	346 (37.1)	587 (62.9)	933*
Female	13 (34.2)	25 (65.8)	38
Opiate positive	169 (29.8)	398 (70.2)	567*
Opiate negative	14 (25.5)	41 (74.5)	55
Median age (years)	29.7	24.0	25.9†
≤ 26 years old	165 (22.7)	563 (77.3)	728**
> 26 years old	156 (48.2)	168 (51.8)	324
Year of first HIV seropositivity			
1987-1990	121 (82.3)	26 (17.7)	147
1991-1994	85 (51.1)	89 (48.8)	174
1995-2000	225 (25.8)	646 (74.2)	871‡

Note: *Chi-square test, $p > 0.05$; **Chi-square test, $p < 0.05$;
† t test, $p < 0.05$; ‡Chi-square test for trend, $p < 0.05$

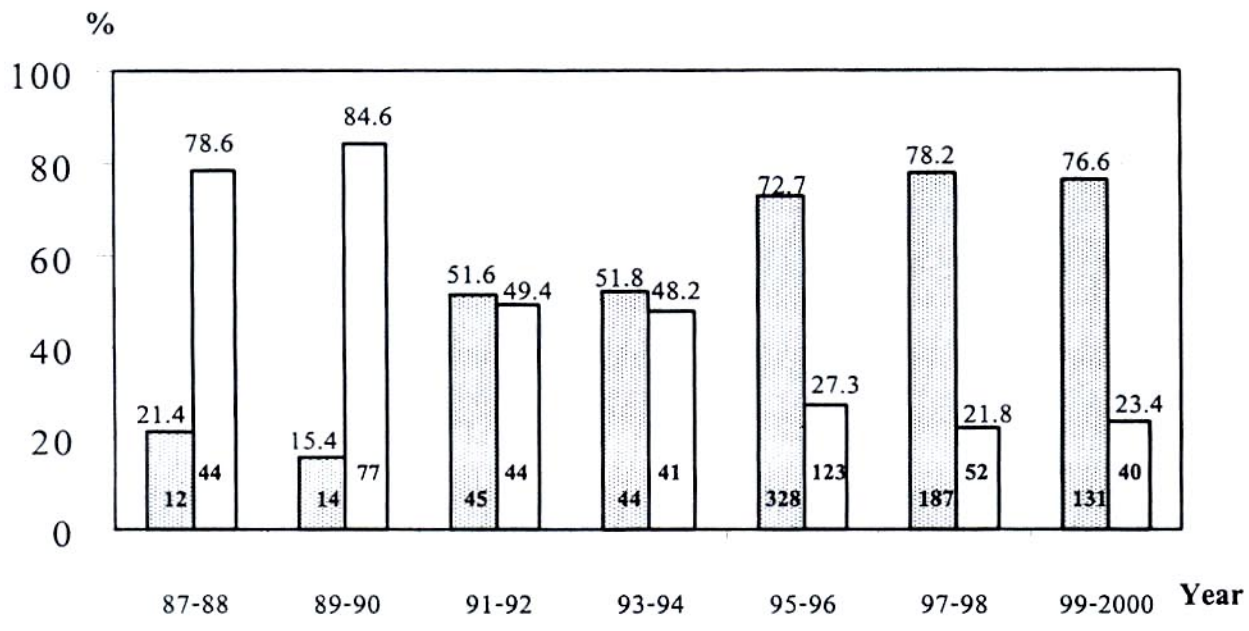

Fig. 1 Proportion of HIV-1 subtype E (■) and B′ (□) by year of first HIV seropositivity. The number inside each column represents the absolute number of each subtype. The number on top of the column represents the percentage of each subtype.

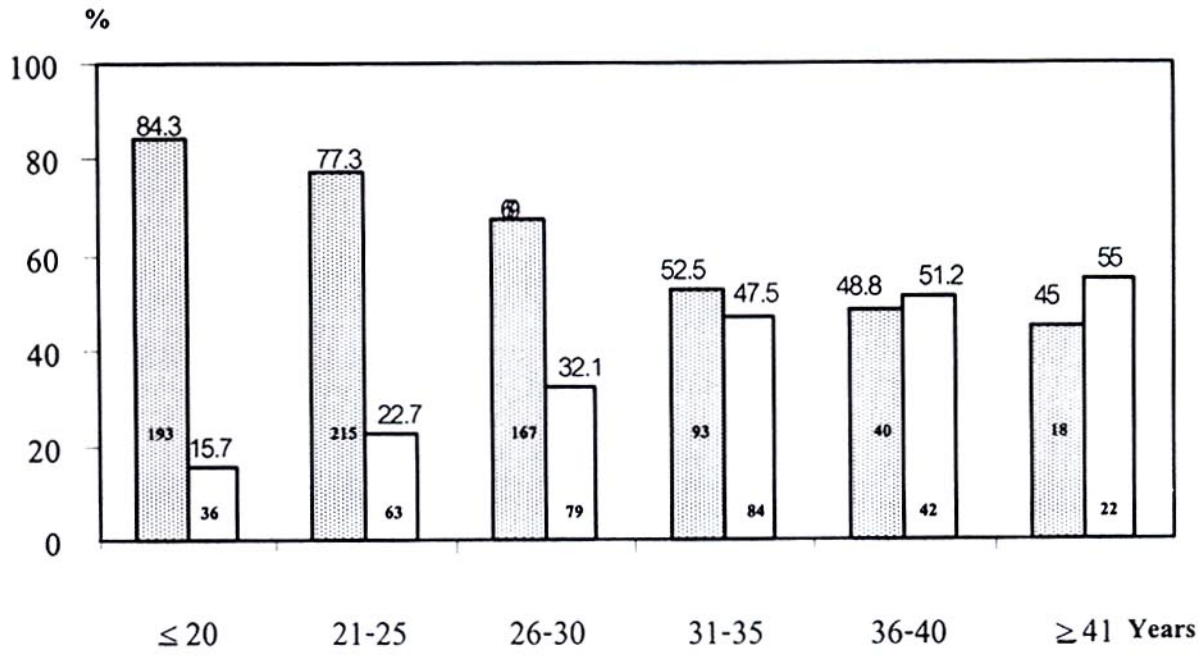

Fig. 2 Proportion of HIV-1 sybtype E (▨) and B' (□) by age at first HIV seropositivity. The number inside each column represents the absolute number of each subtype. The number on top of the column represents the percentage of each subtype.

From Fig. 1, it can be seen that the proportions of HIV-1 subtype B′ decreased from 78% during 1987 and 1988, to 48% during 1993 and 1994, and then to 23% during 1999 and 2000. The declines were statistically significant ($p < 0.05$ by Chi-square test for trend). It should be observed that even early in the HIV epidemic, i.e. during 1987 and 1988, the proportion of HIV-1 subtype E among the IDU was as high as 21%.

HIV subtypes and age at first HIV seropositivity

As shown in Fig. 2, the proportions of subtype B′ decreased as the age at first HIV seropositivity increased over time. At the same time, the proportions of subtype E increased. The changes were statistically significant ($p < 0.05$ by Chi-square test for trend).

DISCUSSION

Injecting drug users have been a well-recognized risk group for HIV infection and they have been a subject of extensive studies including virological, behavioral, clinical, demographic and cohort studies for vaccine trials.[17-19] HIV subtypes B′ and E were recognized by McCutchan *et al.*[14] and Ou *et al.*[7] in 1992 with specimens collected in 1990 and 1991, respectively. The former reported that 4 out of 5 (80%) parenterally infected Bangkok residents were of subtype B′ while the latter demonstrated that 22 out of 29 (76%) HIV-infected IDU were of subtype B′.

Our study provides additional data on HIV-1 subtype distribution among IDU in central Thailand. The retrospective data enable us to observe levels and changes of HIV-1 subtypes distribution from 1987 to 2000. Kalish *et al.*[15] and Pau *et al.*[12] reported that 83% and 76%, respectively, of newly-infected IDU during 1988-1993 were of subtype B′. The data are comparable to what we observed in this study during the same period. In addition, the proportions of HIV subtype B′ during 1990 and 1991 as reported by McCutchan *et al*[14] and Ou *et al*[7] in 1992 compared favorably with our finding.

Despite high proportions of subtype B′ in earlier years, the proportions decreased rapidly over years with corresponding increases

in proportions of subtype E. Wasi *et al.*[9] reported the proportion of subtype E among newly infected IDU in Bangkok to be 2.6% during 1988 and 1989, and to increase to 25.6% and 56.2% during the 1990-1991 and 1992-1993 periods, respectively. Our findings support the rapid increase in the proportion of subtype E, except that the proportion of subtype E during the 1988-1989 period in our study was much higher than that reported by Wasi *et al.*[9] (21.4% *vs* 2.6%). Several other studies also confirmed the rapid rise of subtype E proportion.[10,11,15,16]

The much higher proportion of HIV-1 subtype E among infected IDU during 1987-1990 as found in this study warrants further investigation. This indicates that subtypes B′ and E co-existed since early in the epidemic. Therefore, if the two subtypes were introduced independently and tended to segregate by different transmission routes, it is likely that subtype E was introduced into the IDU population soon after subtype B′ and subtype E caught up quite quickly. There are several theoretical possibilities why subtype E could catch up so quickly, e.g. transmission via more efficient route(s), transmission through more prevalent practice(s), a higher compatibility of subtype E with the hosts.

We observed differences in the HIV subtype distribution by age. It was found that younger HIV-infected IDU tended to have a higher proportion of subtype E than B′. This finding is consistent with other studies.[10,16] The higher proportion of subtype E among the youth needs further considerations of some potential explanations. First, younger IDU tend to be more sexually active. Therefore, if subtype E has a higher transmissibility via sexual routes than subtype B′, this could explain the different proportions of the two subtypes. Whether this is the case needs further study. Second, it is possible that IDU who are infected with subtype B′ tend to be sicker, develop more serious illnesses, and die more quickly. Therefore, even if the IDU would be infected equally with the two subtypes, there would be fewer IDU living with subtype B′ left, as compared to the number of IDU left living with subtype E. Available evidences suggest that there are no differences in the spectrum of opportunistic infections, level of immunosuppression, and mortality rates between the two groups.[11,18-19] However, further investigations are needed.

The role of sexual transmission in explaining the higher proportion of HIV subtype E among young IDU cannot be underestimated and this highlights the importance of more research on sexual risk factors among IDU, especially among the youth. The research should focus on the relative importance of different routes, i.e. drug injection *versus* sexual practice, of HIV transmission in the young IDU.

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