Original Article

Substantially Exposed but HIV-Negative Individuals Are Accumulated in HIV-Serology-Discordant Couples Diagnosed in a Referral Hospital in Thailand

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SUMMARY: The objective of this study is to characterize HIV-serology-discordant couples diagnosed at a referral hospital in Thailand and to identify risk factors for HIV transmission among married couples. Firstly, cross-sectional analysis was conducted from July 2000 to October 2002. Out of 216 HIV-positive married men who knew the HIV status of their wives, the median number of sexual contacts in 63 men with HIV-negative wives was 6 times per month before the disclosure of HIV status, which did not differ from 153 men with HIV-positive wives. The majority of men with HIV-negative wives never used condoms. The median duration of marriage was 7 years for both groups. Unlike in previous reports, men with HIV-negative wives were significantly more symptomatic ($P < 0.01$), and their CD4+ counts and viral loads did not differ from men with HIV-positive wives. Secondarily, 71 initially discordant couples were longitudinally followed until March 2005. Four were seroconverted out of 132.24 person-years of observation. In multivariate analysis incorporating sex, age, CD4+ count and sexual contact without a condom, shorter duration of marriage (<2 years) was found to be the only risk factor significantly associated with HIV transmission (hazard ratio of 15.2, $P = 0.04$). Individuals substantially exposed to HIV but remaining HIV-negative are accumulated in discordant couples identified in a hospital, except in recently married couples.

INTRODUCTION

Thailand was the first country to be severely affected by the human immunodeficiency virus (HIV) epidemic in Asia. Its epidemic is characterized by heterosexual intercourse being the predominant mode of HIV transmission. Commercial sex workers (CSWs) served as a core group and spread the virus rapidly to male clients; the male clients transmitted it to their wives, who then transmitted it to new born babies. The 100% condom campaign succeeded in dramatically slowing the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced the spread of HIV by brothel-based CSWs, and the prevention programs for mother-to-child HIV transmission reduced. CCR5, HLA, DC-SIGNR. This indicates that the susceptibility to HIV infection is also genetically determined (6-8).

These risk factors for HIV transmission among married couples differ in a population with a different genetic, behavioral, and cultural background. For instance, circumcision is not a common practice in Thailand, and CCR5 30 nucleotides deletion is very rare (9). There has been only one study from Asia which investigated married couples to assess the risk factors for HIV transmission (10-12). This study identified HIV-discordant couples by actively recruiting spouses of HIV-positive partners who were diagnosed at a hospital when HIV-positive spouses develop HIV-related symptoms. We conducted this study with two objectives: (i) to characterize HIV-serology-discordant and concordant couples who were diagnosed at a referral hospital in Thailand, and (ii) to identify risk factors for HIV transmission among those HIV-serology-discordant couples.
Materials and methods

Study population: We conducted a hospital-based study at the day care center of Lampang Hospital in northern Thailand. The Lampang Hospital is a government referral hospital with approximately 800 beds, and it is situated in the central district of Lampang province to cover nearly one million people in Lampang province. From 6 July 2000 to 15 October 2002, a study coordinator approached every patient attending the HIV clinic at the day care center and encouraged them to bring their spouses to participate in this study. Participants were interviewed separately by trained interviewers, who obtained sociodemographic data and marital status. Two designated research clinicians obtained clinical data by conducting a physical examination and collected information concerning history of STDs, frequency of sexual contacts per week and the use of condoms with their partners. All participants gave written informed consent. The study procedure and consent form were reviewed and approved by the ethical review committee of the Thai Ministry of Public Health in December 1999.

Follow-up procedure for HIV-negative spouses: We followed up HIV-negative spouses by testing them every 6 months for anti-HIV serology. Free condoms were provided every 3 months at the day care center throughout the study period. The last date of serological testing was 2 March 2005. If the HIV-positive spouse died, or if the couple separated or divorced, we repeated the serology test one more time at least 1 month after the death or separation, and then stopped the follow-up. The duration of follow-up was calculated from the date of study enrollment to either the last date of serological testing if couples remained living together, or to the date of death or divorce for index cases. When the HIV serology negative spouse seroconverted, the midpoint between the last date with an anti-HIV negative result and the first date with an anti-HIV positive result was taken as the date of seroconversion.

Laboratory tests: HIV infection was determined by the detection of anti-HIV antibodies by enzyme-linked immunosorbent assay (ELISA) (Enzynost anti HIV-1/2 plus; Dade Behring Marburg GmbH, Marburg, Germany). Positive results were confirmed by a subsequent ELISA test (Genscreen HIV 1/2 Diagnostic; Pasteur Ltd., Paris, France) and a gel particle agglutination test (Serodia HIV-1; Fujirebio Inc., Tokyo, Japan). Serum levels of HIV-1 RNA were quantified by a reverse transcription-polymerase chain reaction (RT-PCR)-based assay (Amplicor HIV-1 monitor 1.5 assay; Roche Molecular System, Branchburg, N.J., USA), with a lower detection limit at 400 copies/ml. The CD4+ cell count was measured by a standard flow cytometric technique using FACScan (BD Biosciences, San Jose, Calif., USA) with fresh EDTA-treated whole blood at the time of bleeding.

Data management and statistical analysis: Continuous variables were compared by a non-parametric test, the Kruskal-Wallis test. Discrete data were compared by the chi-square test or Fisher’s exact test, as appropriate. Survival analysis was conducted by the Cox proportional hazard model. Statistical analysis was performed using the software Stata, version 6.0.

Results

Study population: Seven hundred fifty-six HIV-positive cases and 71 HIV-negative spouses living with an HIV-positive spouse were enrolled. This represents over 97% of HIV-positive individuals who attended the clinic during the study period. Of the 756 HIV-positive cases, 320 were men, of whom 262 (81.9%) had ever been married. Among those men who reported having been married, 153 (58.4%), 63 (24.0%), and 46 (17.6%) reported that the HIV status of their wives was positive, negative and unknown, respectively. There were 436 HIV-positive women, of whom 435 were married: 354 (81.4%), 34 (7.8%), and 47 (10.8%) reported that the HIV status of their husbands was positive, negative, and unknown, respectively. The proportion of HIV-positive men having HIV-negative wives was significantly higher than that of HIV-positive women having HIV-negative husbands (63/262 [24%] versus 34/435 [7.8%], P < 0.0001). The proportion of remarried cases was higher among HIV-positive women with HIV-negative husbands, compared with HIV-positive women with HIV-positive husbands (24/34 [71%] versus 113/354 [31.9%], respectively, P < 0.0001). This is because the former group contained more cases, in which widows of an HIV-positive ex-husband remarried.

Characteristics of HIV-positive men who have HIV-negative wives versus HIV-negative wives: We compared the characteristics of HIV-positive men according to the HIV status of their most recent wives (Table 1). We found no significant differences in age, transmission route, history of anti-HIV drug use and other previously reported risk factors such as history of STD, CD4+ cell count, viral load, and frequency of sexual contacts per month before and after the disclosure of HIV infection among the spouses. However, we found that men with HIV-negative wives were more likely to be symptomatic than men with HIV-positive wives (P < 0.001). The proportion of men reporting 100% condom use before the disclosure of HIV infection was significantly higher among men with HIV-negative wives than men with HIV-positive wives (3/58 [5.2%] versus 6/128 [3.3%, P < 0.01]. However, the majority (46/58, 79.3%) of men with HIV-negative wives reported having never used a condom. After the disclosure of HIV infection, the frequency of sexual contact declined drastically, and the use of condoms increased in both groups while the significant difference in the frequency of condom use disappeared between the two groups.

HIV incidence rates in HIV-serology-discordant couples: Of 71 HIV-serology-discordant couples, follow-up data were available for 63 (88.7%) HIV-negative spouses: 25 HIV-negative men and 38 HIV-negative women. The median (IQR) follow-up duration was 764 (172, 1,344) days and the total observation period was 132.24 person-years of observation (PYO). Of the 63 couples, follow-ups of 4 HIV-negative men and 18 HIV-negative women were stopped before the census date because their spouses had died (4 men and 17 women) or due to divorce (1 woman). There were 4 seroconversion cases, resulting in an HIV incidence rate of 3.02 seroconversions/100 PYO with a 95% confidence interval (95% CI) of 0.06 to 5.98 seroconversions/100 PYO.

Characteristics of HIV-serology-discordant couples with subsequent seroconversion: To determine the risk factors associated with HIV seroconversion in this discordant couple cohort, we analyzed the characteristics of HIV-discordant couples with or without subsequent HIV transmission (Table 2). We found that the median duration of mar-
riage was significantly shorter among discordant couples with HIV seroconversion compared with the non-seroconverters.

A higher proportion of those who seroconverted reported having sex without condoms (3/4, 75%) compared to those who remained seronegative (16/59, 27.1%; \( P = 0.078 \) by Fisher’s exact test). This tendency was maintained throughout despite our efforts to provide counseling and supply condoms during the study period. The HIV seroconverters reported more frequent sexual contacts than the non-seroconverter couples, but the difference was not significant. Those who seroconverted tended to be male and younger, but none of these differences were statistically significant. Interestingly, HIV-positive index cases of HIV seroconverters were significantly more likely to be asymptomatic (\( P = 0.01 \)), and they tended to have a high CD4+ cell count and a lower viral load.

**Risk factors for HIV seroconversion:** We then analyzed the risk factors for HIV seroconversion with multivariate analysis using the Cox proportional hazard model incorporating sex, age, marital duration, CD4+ cell count, and any sexual contact without a condom as variables (Table 3). CD4+ cell count was incorporated into the model instead of clinical

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-positive (n = 4)</th>
<th>HIV-negative (n = 58)</th>
<th>Unknown (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.5 [25.5, 29.5]</td>
<td>32 [29, 36]</td>
<td>5.5 [1, 3]</td>
</tr>
<tr>
<td>Marital duration (y)</td>
<td>0.5 [&lt;1, 3]</td>
<td>6 [3, 9]</td>
<td>0.01</td>
</tr>
<tr>
<td>Factors of index cases</td>
<td>Symptomatic</td>
<td>0 (0%)</td>
<td>37 (64.9%)</td>
</tr>
<tr>
<td>CD4+ cells count/μl</td>
<td>284.5 [110, 493]</td>
<td>66 [22, 399]</td>
<td>0.52</td>
</tr>
<tr>
<td>History of STD</td>
<td>0</td>
<td>18</td>
<td>0.19</td>
</tr>
<tr>
<td>History of ARV use</td>
<td>0</td>
<td>16</td>
<td>0.22</td>
</tr>
<tr>
<td>Sexual behavior</td>
<td>Frequency of sexual contacts per month</td>
<td>7 [4, 12]</td>
<td>3.25 [1, 8]</td>
</tr>
<tr>
<td>Any sexual contact without condom</td>
<td>Yes</td>
<td>3 (75.0%)</td>
<td>16 (27.1%)</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>43 (72.9%)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Median [Inter-quartile range] is shown;  
1: Clinical information was available only for 63 HIV-positive index cases.  
2: Sexual behavior after the disclosure of HIV status is shown.  
STD, sexually transmitted diseases; ARV, antiretroviral drug.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Univariate analysis Hazard ratio [95% CI]</th>
<th>Multivariate analysis Hazard ratio [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Being male</td>
<td>3.36</td>
<td>0.30</td>
</tr>
<tr>
<td>Age</td>
<td>8.53</td>
<td>0.06</td>
</tr>
<tr>
<td>&lt;30 years old</td>
<td>0.88</td>
<td>0.04</td>
</tr>
<tr>
<td>Marital duration</td>
<td>12.86</td>
<td>0.03</td>
</tr>
<tr>
<td>CD4+ cells count/μl</td>
<td>3.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Any sexual contact without condom</td>
<td>6.00</td>
<td>0.12</td>
</tr>
</tbody>
</table>

CI, confidence interval.
symptoms, since none of the index cases of HIV seroconverters were symptomatic, and the CD4+ cell counts were closely associated with the clinical status of index cases ($P < 0.001$). Only the duration of marriage (less than 2 years) was significantly associated with HIV seroconversion ($P = 0.027$), and younger age (less than 30 years) was marginally associated in the univariate analysis ($P = 0.064$). This trend did not change when multivariate analysis was conducted. Kaplan-Meier survival curves stratified by the duration of marriage are shown (Figure 1). HIV seroconversion was significantly more frequent among HIV-negative spouses with a marital duration of <2 years than the other group. The figure also demonstrates the proportionality of the survival curves between the two groups.

**DISCUSSION**

Preventing HIV transmission in discordant married couples remains a challenging issue. To improve counseling and intervention measures in married couples, it is useful to identify a particular risk group for HIV transmission so that we can target our measures where they are most needed. This is the first study to investigate the characteristics of HIV-discordant couples and analyze risk factors for HIV transmission in a hospital setting in Asia. In contrast to previous publications, we did not find any significant association of HIV transmission with a high viral load, a low CD4+ cell count, the presence of clinical symptoms or a longer duration of relationship. Instead, our study showed the opposite results that men with HIV-positive wives were significantly less likely to be symptomatic than men with HIV-negative wives, and that HIV-positive index cases of HIV-negative spouses who subsequently seroconverted tended to have a lower viral load and a higher CD4+ cell count. Furthermore, we found that a shorter duration of marriage was the only significant risk factor associated with subsequent HIV transmission in this small-scale discordant couple cohort. This finding was not reported previously.

We believed that the main reason for this discrepancy was the difference between our study populations and those from previous studies. Our study subjects were recruited at the HIV clinic of a referral hospital, whereas previous studies were conducted in a community or at a blood bank (10-15). With the exception of HIV-positive pregnant women who were identified during prenatal HIV screening, HIV-positive index cases recruited in a hospital setting were diagnosed at an advanced stage of HIV infection because they visited the hospital as they developed HIV-related symptoms. In fact, the majority of HIV-positive index cases were symptomatic in our hospital-based study, whereas the majority of index cases in a previous study done at a blood bank were asymptomatic and in an early stage of HIV infection (10-12). The Nairobi CSW cohort showed that the incidence rate of HIV-1 seroconversion decreased with increasing duration of exposure, which indicates that those who remain HIV-negative for a longer period were more likely to be resistant to HIV infection (16). The difference in the slopes of the survival curves according to the duration of marriage shown in Fig. 1 is compatible with this Nairobi CSW model. Those HIV-negative spouses who were recently married showed a faster decline, and all index cases were asymptomatic. We presume that the majority of discordant couples identified in a non-hospital-based study are similar to such recently married couples. On the other hand, the majority of HIV-negative spouses of HIV-positive patients identified in a hospital are highly exposed but HIV-negative individuals who carry some biological mechanisms that confer resistance to HIV infection such as anti-HIV immunity or host-genetic polymorphisms (6,7,17,18).

Our results showed a dramatic change in sexual behavior before and after the diagnosis of HIV infection. However, despite our effort to provide counseling and condoms, four seroconverters were found as discordant couples were followed up in our study. Furthermore, none of the four HIV-seropositive index cases of seroconverters had any HIV-related symptoms. It was also observed that HIV-positive index cases with HIV-positive wives were significantly more likely to be asymptomatic than those with HIV-negative wives. We hypothesized that the absence of clinical symptoms may have led to more risky sexual behavior. When we analyzed this relation, we found that if index cases were symptom-free, they tended to have unprotected sex with their spouses, but this relation was not significant, probably due to the small sample size (data not shown). Because of the small sample size and the limited number of seroconversions, the CI of hazard ratios was very wide, and we have to interpret the results with caution. However, while we cannot over-stress the importance of counseling all HIV-discordant couples, our results may indicate that HIV-negative spouses who are recently married to asymptomatic index cases are at a particularly high risk of HIV infection.

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