CONCISE COMMUNICATIONS

Molecular Methods for the Diagnosis of Genital Ulcer Disease in a Sexually Transmitted Disease Clinic Population in Northern Thailand: Predominance of Herpes Simplex Virus Infection


A multiplex polymerase chain reaction (M-PCR) assay that simultaneously detects the three major causes of genital ulcer disease (GUD), Haemophilus ducreyi, Treponema pallidum, and herpes simplex virus, was used to evaluate swab specimens for 38 sequential patients with GUD at a Thai sexually transmitted disease clinic. Subjects received clinical diagnoses and syndromic treatment. Swab specimens for H. ducreyi cultures and M-PCR were obtained. No H. ducreyi cultures were positive. Of 38 M-PCR specimens, 31 (81.6%) were positive for HSV, 1 (2.3%) for both HSV and T. pallidum, and none for H. ducreyi or T. pallidum alone; 6 (15.8%) were negative for all 3 pathogens. Clinical diagnoses corresponded poorly to M-PCR findings; none of 5 suspected cases of chancroid were positive by M-PCR and none of 1 for syphilis, but 21 of 24 suspected herpes lesions were confirmed by M-PCR. Human immunodeficiency virus infection status was known for 24 of 38 subjects; 11 (45.8%) were seropositive, and all 11 had HSV by M-PCR. HSV appeared to be the most common pathogen overall.

Genital ulcer disease (GUD) is an important health problem in many developing country settings. GUD has been implicated in several studies as an important factor in the sexual spread of human immunodeficiency virus type 1 (HIV-1) in numerous developing countries, including Thailand [1]. Clinical diagnoses of 3 of the most common etiologic agents of GUD (Haemophilus ducreyi, Treponema pallidum, and herpes simplex virus) in sexually transmitted disease (STD) clinic settings are problematic, however [2]. H. ducreyi is a fastidious organism; while culture is the reference standard for diagnosis, sensitivity of culture varies considerably [3], and culture is not routinely done in Thai Ministry of Public Health (MOPH) clinics. Herpes simplex virus (HSV) can be definitively diagnosed by culture, but cost has limited the utility of this assay in most developing country settings, including Thailand, where viral culture is not used in government STD clinics. HSV ELISAs are also not currently used in Thai MOPH clinics. T. pallidum cannot be cultured with methods available to the clinician and is typically diagnosed either serologically or with darkfield microscopy [3]. However, the latter is also unavailable in many developing country settings, and the sensitivity of serologic assays in acute infection can be as low as 30% [4].

Recently, a multiplex polymerase chain reaction (M-PCR) assay with colorimetric detection has been developed for the simultaneous amplification of DNA targets of these three pathogens using a single swab from genital ulcer secretions [5]. This assay has been used in several studies, including one in Lesotho, where its sensitivity compared with culture for H. ducreyi and HSV was 95% and 93%, respectively [2]. In a large GUD patient series in New Orleans, the sensitivities of M-PCR for HSV, H. ducreyi, and T. pallidum were 100%, 98.4%, and 91%, respectively, versus 71.8% for HSV culture, 74.2% for H. ducreyi culture, and 81% for darkfield microscopy for T. pallidum [5].

Chiang Mai City has been one of the epicenters of the Thai HIV epidemic, with HIV prevalence rates among sex workers reaching 40% by 1990 [6]. HIV prevalence among male STD clinic attenders has ranged from 9% to 20% in MOPH sentinel surveys [7]. In both populations, lifetime STD rates have been found to be extremely high: >80% in sex workers, and >40% in adult men [8]. Since 1989, however, Thailand has engaged in an intensive national campaign to control STDs, reduce HIV...
incidence, and promote condom use in commercial sex settings through its “100 Percent Condom Campaign” [9]. The results of this effort have been remarkable and well documented [9, 10]. STD rates among attenders at government STD clinics fell steadily from 1989 to 1993, from 199,048 reported cases among men in 1989 to 38,835 reported cases in 1993 [9]. The reporting system for STDs included 5 diagnoses: syphilis, gonorrhea, chancroid, lymphogranuloma venereum, and nongonococcal urethritis [9]. During 1989–1993, reported syphilis cases among men attending government STD clinics declined from 11,487 nationwide to 3645, and reported chancroid cases declined from 29,675 to 1990 [9]. The great majority of men diagnosed during this period reported sex worker contact as the source of their STDs [9]. Incident STD rates in several well-characterized cohorts of HIV and STD at-risk groups have confirmed these findings and suggest that treatable, bacterial STDs are becoming uncommon, even in high risk groups [11]. Genital ulcers, however, continue to be reported by clinicians, and remain highly correlated with HIV infection in northern Thailand [12]. Identification of the etiologic agents involved, however, has been limited.

To further investigate the etiology of GUD in northern Thailand, we compared the clinical diagnosis of GUD with *H. ducreyi* culture and with M-PCR in patients attending an urban STD clinic in 1995–1996. Thirty-eight sequential patients presenting over a 4-month period with genital ulcers were managed according to standard clinic practice, which included syndromic management based on clinical findings, and each had 2 additional swab specimens collected from their genital lesions. The study was done under the auspices of STD surveillance by the Office of Communicable Disease Control, Region 10, Chiang Mai, and was approved by the Royal Thai Ministry of Health.

**Subjects and Methods**

The women (n = 30) in the study were sex workers active in Chiang Mai City who attended a weekly clinic for sex workers at the Communicable Disease Control STD Clinic (STD-10) in Chiang Mai. The men (n = 8) were attenders at a men’s STD clinic housed at the same site. HIV and syphilis serologic test results were available for some members of both groups. The STD-10 clinic is the largest such facility in Chiang Mai City and is government-run. Male clinic attenders are generally poor or working class men who cannot afford private clinics. The female clinic attenders represent the bulk of local sex workers, except those in the highest income groups, “indirect,” or non–brothel-based sex workers, who typically use private clinics.

All subjects had a swab specimen collected from their genital lesions for culture of *H. ducreyi*. Specimens were inoculated on activated charcoal agar medium and transported within 2 h to the Research Institute for Health Sciences, Chiang Mai University. Plates were incubated in a moist chamber at 33°C with 10% CO₂ for 48 h. Strains of *H. ducreyi* isolated in Thailand and Africa (courtesy of Centers for Disease Control and Prevention [CDC], Atlanta) were inoculated on the same day as the clinical specimens for quality control. A second swab specimen for M-PCR was agitated in 1 mL of specimen transport medium (Amplitaq; Perkin-Elmer, Norwalk, CT) was used [5]. PCR inhibition was analyzed by use of an internal control plasmid. Specimens were considered positive if both duplicate wells produced signals of A₄₅₀ ≥ 0.25, as has been previously described [5].

**Results**

Of 38 subjects, 8 were male STD patients, and 30 were female sex workers. All had GUD on inspection or pelvic examination. No *H. ducreyi* cultures were positive; control cultures inoculated on the day each clinical specimen was inoculated were all positive. Of 38 specimens, 31 (81.6%) were positive for HSV alone, 1 (2.3%) was positive for both HSV and *T. pallidum*, none were positive for *H. ducreyi* or *T. pallidum* alone, and 6 (15.8%) were negative for all 3 pathogens (table 1). HIV status was available for 24 of 38 subjects (4 men, 20 women); of these, 11 (45.8%) of 24 were known to be HIV-seropositive; 2 of 4 men, and 9 of 20 women. All 11 HIV-seropositive persons were M-PCR–positive for HSV but for no other pathogen. Among the 13 known HIV-seronegative persons, a lower, but not statistically significant percentage (77%, 10/13) were M-PCR–positive for HSV (table 1).

Clinical diagnoses corresponded poorly with M-PCR findings for chancroid and syphilis; none of 6 suspected cases of chancroid were positive by either culture or M-PCR, and 0 of 1 for syphilis. Of the 6 clinically suspected cases of chancroid, 1 was negative for all pathogens by M-PCR and 5 were positive for HSV. Of 24 clinically suspected cases of genital herpes, 21 (87.5%) were confirmed by M-PCR. Among the men, 6 of 8 had herpes simplex infection by M-PCR, and the remaining 2 had negative results for all 3 pathogens. Clinical correlation with M-PCR findings did not vary significantly by HIV status; 6 (55%) of 11 HIV-seropositive persons had a clinical diagnosis in agreement with M-PCR results, as did 8 (62%) of 13 HIV-seronegative persons.

**Discussion**

HSV was the most common cause of genital ulceration in this sample, accounting for the large majority of positive findings. Since both syphilis and chancroid infections can be cured with antimicrobial agents that are widely available and used in Thailand, the relative scarcity of these infections in the high-risk population we studied suggests that these
Table 1. Multiplex polymerase chain reaction (M-PCR) results and clinical diagnoses for 38 sequential genital ulcer patients evaluated at Chiang Mai City STD Clinic, Chiang Mai, Thailand, July–October 1995.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Ulcer location, description</th>
<th>Clinical diagnosis</th>
<th>HIV status</th>
<th>M-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>F</td>
<td>Perineal, single, discharge</td>
<td>Chancroid T. vaginalis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>05</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>F</td>
<td>Labia majora, single</td>
<td>Chancroid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>F</td>
<td>Labia minora</td>
<td>Chancroid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>Labia majora, multiple, discharge</td>
<td>Chancroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>Labia minora, erythema, discharge</td>
<td>U, GUD</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>Labia majora, multiple, pain, discharge</td>
<td>Chancroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>Labia majora, multiple, firm</td>
<td>Severe HSV</td>
<td>+</td>
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</tr>
<tr>
<td>18</td>
<td>F</td>
<td>Perineal, multiple, pain</td>
<td>HSV</td>
<td>NA</td>
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</tr>
<tr>
<td>19</td>
<td>F</td>
<td>Labia majora, pain, discharge</td>
<td>Chancroid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>F</td>
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<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>HSV</td>
<td>+</td>
<td></td>
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<tr>
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<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
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<tr>
<td>23</td>
<td>F</td>
<td>Labia majora</td>
<td>HSV</td>
<td>+</td>
<td></td>
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<tr>
<td>24</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>HSV, bacteria</td>
<td>+</td>
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</tr>
<tr>
<td>26</td>
<td>M</td>
<td>Glans penis, pain</td>
<td>HSV</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>Glans penis, pain</td>
<td>HSV</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>Glans penis, pain</td>
<td>Balanitis</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>Glans penis, pain</td>
<td>Psoriasis, rule out syphilis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>Labia minora, periurethral, multiple</td>
<td>HSV, syphilis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>U</td>
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</tr>
<tr>
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<td>F</td>
<td>Labia majora, multiple</td>
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<td>NA</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>Glans penis</td>
<td>U</td>
<td>+</td>
<td></td>
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<tr>
<td>34</td>
<td>M</td>
<td>Glans penis</td>
<td>HSV</td>
<td>NA</td>
<td></td>
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<tr>
<td>35</td>
<td>F</td>
<td>Labia majora, multiple vesicular</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>M</td>
<td>Glans penis</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>M</td>
<td>Glans penis, painless</td>
<td>Syphilis, mixed infection</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>F</td>
<td>Labia majora, multiple</td>
<td>HSV</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Total 11/24 32 0 1

NOTE. HD, Haemophilus ducreyi; TP, Treponema pallidum; NA, HIV status not available at time of study; U, = uncertain clinical diagnosis.

Infections may be well controlled in this region. This finding is in accord with recent reports from Thai national STD clinic data showing sharp declines in reported syphilis, chancroid, and treatable inflammatory STDs, including gonorrhea and nongonococcal urethritis [9]. These trends are further supported by epidemiologic studies that have documented recent declines in individual STD acquisition behaviors, increasing condom use, and decreasing self-reported STD episodes in northern Thailand [10, 13]. Since HSV is a chronic infection that cannot be cured with current treatments, we would not expect to see similar short- and medium-term declines in the prevalence of this infection with population-level STD treatment programs. However, the high rates of condom use in commercial settings in Thailand reported by several groups may have an effect on HSV transmission rates and may lead to delayed declines in the prevalence of this infection as well [10, 12]. Antibiotics are widely available without prescription in Thailand, and self-treatment for STDs is common; >60% of men in 2 populations studied by our group in northern Thailand.
reported attempts at self-treatment before seeking medical attention for an STD [7]. These behaviors may have impacted the low rate of positive cultures for chancroid in this STD patient series. However, the finding that >80% of GUD patients did have a positive M-PCR for HSV suggests that bacterial STDs may indeed be uncommon. The rate of M-PCR–negative specimens (6/38, 15.8%) is similar to that reported in other studies [5]. There are several possible explanations for these M-PCR–negative specimens, including ulcers due to trauma, low target organism numbers, inadequate sampling techniques, and lesions due to other etiologic agents. These findings suggest a potential use of M-PCR for STD surveillance; if larger samples confirmed the low prevalence of chancroid, approaches to clinical care in this setting could be revised accordingly, and unnecessary empiric treatment for bacterial STDs could be reduced.

It is not surprising that clinical findings correlated poorly with M-PCR results; clinical manifestations of all three infections studied can vary significantly [14]. In addition, concurrent HIV infection may complicate clinical diagnoses of both HSV and syphilis, although in this small sample this did not appear to be the case. The HIV prevalence among persons attending the study clinic for sex workers was 40%–60% during the months of the study, and so the finding of 45.8% HIV seroprevalence, while high, is not unexpected. The finding that 11 of 11 of HIV-seropositive persons were M-PCR–positive for HSV underscores the need for continued safer sex education and condom use promotion to prevent spread of both of these infections in this population.

This sample of sex workers and male STD clinic attenders, although too small to generalize to the population at large, is further evidence to suggest that the Thai national mobilization to control STDs may be working for bacterial causes of GUD. Continued high rates of condom use, however, will be necessary to decrease the transmission of HIV and HSV.

References