Chancroid, Primary Syphilis, Genital Herpes, and Lymphogranuloma Venereum in Antananarivo, Madagascar

Frieda M.-T. Behets,1,2 Jocelyne Andriamiadana,3 Daudet Randrianasolo,3 René Randriamanga,4 Désiré Rasamialao,3 Cheng-Yen Chen,4 Judith B. Weiss,5 Stephen A. Morse,4 Gina Dallabetta,6 and Myron S. Cohen1

1Department of Medicine, University of North Carolina at Chapel Hill; 2Yale School of Public Health, New Haven, Connecticut; 3Institut d’Hygiène Sociale, Ministry of Health, Antananarivo, Madagascar; 4Centers for Disease Control and Prevention, Atlanta, Georgia; 5Roche Molecular Systems, Alameda, California; 6AIDS Control and Prevention Project, Family Health International, Arlington, Virginia

Ulcer material from consecutive patients attending clinics in Antananarivo, Madagascar, was tested using multiplex polymerase chain reaction (M-PCR) to detect Treponema pallidum, Haemophilus ducreyi, and herpes simplex virus. Sera were tested for syphilis and for IgG and IgM antibodies to Chlamydia trachomatis by microimmuno­fluorescence testing (MIF). By M-PCR, 33% of 196 patients had chancroid, 29% had syphilitic ulcers, and 10% had genital herpes; 32% of the ulcer specimens were M-PCR negative. Compared with M-PCR, syphilis serology was 72% sensitive and 83% specific. The sensitivity of clinical diagnosis of syphilis, chancroid, and genital herpes was 93%, 53%, and 0% and specificity was 20%, 52%, and 99%, respectively. Less schooling was associated with increased prevalence of syphilitic ulcers (P = .001). Sixteen patients (8%) were clinically diagnosed with lymphogranuloma venereum (LGV); 1 plausible case of LGV was found by MIF. In Madagascar, primary care of genital ulcers should include syndromic treatment for syphilis and chancroid.

While human immunodeficiency virus (HIV) remains relatively rare in Madagascar [1], a few published studies and anecdotal reports suggest that the prevalence of treatable sexually transmitted diseases (STDs) is high. Surveys conducted in 1995 in Antananarivo, Toamasina, and Tulear showed that 12% of women attending prenatal clinics and 30% of nonregistered prostitutes were syphilis seroreactive, whereas HIV seroprevalence was lower than 0.5%, even in the highest risk groups [2]. One study attempted to determine the etiology of genital ulcers at the public STD clinic in Antananarivo, Madagascar [3]. The authors concluded that 56% of 61 persons with genital ulcers had syphilis, 29% had lymphogranuloma venereum (LGV), 20% had chancroid, 2% had herpes simplex virus (HSV), and in 15% the diagnosis was not known.

We conducted a study to determine the etiology of genital ulcers in Antananarivo using a multiplex polymerase chain reaction (M-PCR) assay to detect amplified DNA targets from Haemophilus ducreyi, Treponema pallidum, and HSV in a single ulcer specimen [4]. Because clinicians are often reluctant to change usual practice, we also assessed the accuracy of clinical diagnosis and locally performed laboratory tests for comparison with M-PCR results.

Methods

Consecutive patients seeking primary care in Antananarivo, at the public STD clinic of the Institut d’Hygiène Sociale or at the nongovernmental 67 Ha STD clinic who were ≥18 years old and presented with a complaint of genital ulcer(s), or whose genital ulcer disease (GUD) was discovered during a clinical examination, were asked to participate in the study. Any genital epithelial disruption was considered to be a genital ulcer. Experienced STD care–providing physicians examined the patients. Interviews were conducted using a structured questionnaire. Clinical diagnoses were made by the physicians solely on the basis of the physical examination and history without knowledge of the laboratory results. Material from the clean base of the ulcers was collected using sterile swabs that were expressed into Amplicor specimen transport medium (Roche Molecular Systems). The ulcer specimens were frozen at −20°C until analyzed at the Centers for Disease Control and
Table 1. Multiplex polymerase chain reaction–based etiology of 196 genital ulcers in Madagascar.

<table>
<thead>
<tr>
<th>Etiology of genital ulcers</th>
<th>No. (%) of genital ulcers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemophilus ducreyi</em></td>
<td>61 (31.3)</td>
</tr>
<tr>
<td><em>Treponema pallidum</em></td>
<td>51 (26.2)</td>
</tr>
<tr>
<td>Herpes simplex virus (HSV)</td>
<td>15 (7.7)</td>
</tr>
<tr>
<td><em>T. pallidum</em> and HSV</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td><em>H. ducreyi</em> and <em>T. pallidum</em></td>
<td>2 (1.0)</td>
</tr>
<tr>
<td><em>H. ducreyi</em> and HSV</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>62 (31.8)</td>
</tr>
</tbody>
</table>

Prevention (CDC) using M-PCR (Roche Molecular Systems, Branchburg, NJ) for the detection of *H. ducreyi*, *T. pallidum,* and HSV by techniques described elsewhere [4].

Sera were screened using rapid plasma reagin (RPR; Becton Dickinson, Cockeysville, MD). Reactive sera were tested using *T. pallidum* hemagglutination (TPHA; Fujirebio, Tokyo). Frozen aliquots of sera were sent to the Chlamydia Laboratory, University of California, San Francisco, for chlamydia IgG and IgM antibody detection using microimmunofluorescence (MIF) [5, 6].

Data were entered in a database and analyzed using EpilInfo version 6.02 (CDC, Atlanta) and SAS version 6.12 (SAS Institute, Cary, NC). Differences in proportions were tested by χ² or two-tailed Fisher’s exact test. Means were compared by analysis of variance for normally distributed data; to compare two groups with nonhomogeneous variances determined by Bartlett’s test, the non-parametric Kruskal-Wallis test was used. To calculate sensitivity, specificity, and predictive values of clinical diagnosis, M-PCR results were used as the reference standard. Multivariate analyses were performed using logistic regression. Variables hypothesized to be associated with the outcome in bivariate analyses and potential effects modifiers and confounders were entered into a model. The final model was selected after stepwise elimination of the variables that did not contribute to the fit of the model at the .05 significance level while controlling for confounding (judged to occur when crude and adjusted odds ratios [ORs] differed by ≥10%).

Results

Between 19 March and 30 July 1997, 139 (70.9%) men and 57 (29.1%) women with GUD were evaluated. The study participants were on average aged 26.3 years (median, 25.0 years). Twenty-five (12.8%) of the 196 patients reported <6 years of schooling, 54 (27.5%) reported 6 years, 69 (35.2%) 9 years, 36 (18.4%) 12 years, and 12 (6.1%) had completed higher education. The study subjects stated that the duration of their genital ulcer was, on average, 15.3 days (median, 10); 16.7 days (median, 14.0) for men and 11.5 days (median, 7) for women (P = .026). Of the 73 patients who had already taken some medication for their current episode of GUD, 23 (31.5%) had taken medication prescribed by other health care providers. Women were more likely to have their GUD detected by a physician than were men (OR, 3.78; 95% confidence interval [CI], 1.55–9.23; P = .005). All men were circumcised. Nine (16.7%) of 54 women were pregnant.

The patients presented on average with 2.7 ulcers (median, 2.0); inguinal lymphadenopathy was recorded by the clinicians in 57 (30.0%) of 190 patients. Syphilis and chancroid were clinically diagnosed in 84 (42.9%) of 196 patients, syphilis in 72 (36.7%), chancroid in 14 (7.1%), LGV in 8 (4.1%), syphilis and LGV in 8 (4.1%), scabies in 8 (4.1%), genital herpes in 1 (0.5%), and 1 patient (0.5%) had an unspecified “other” diagnosis. Results of M-PCR testing revealed that 64 (32.6%) of the ulcer specimens contained *H. ducreyi* DNA, 56 (28.6%) *T. pallidum* DNA, 19 (9.7%) HSV DNA, and 62 (31.6%) were M-PCR negative (table 1). Multiple agents were detected in 6 (3.1%) specimens. The sensitivity, specificity, and predictive values of clinical diagnosis compared with M-PCR analysis are reported in table 2.

Patients whose ulcer was caused by *T. pallidum,* as determined by M-PCR, were more likely to have their ulcer detected by a physician during a clinical examination than by themselves (OR, 2.59; 95% CI, 1.07–6.27; P = .05). The prevalence of syphilis did not statistically differ by gender. M-PCR–defined syphilitic ulcers were diagnosed in 5 (55.6%) of 9 pregnant women, compared with 8 (19.0%) of 42 nonpregnant women (OR, 5.31; 95% CI, 0.88–32.35; P = .036). Patients with syphilitic ulcers determined by M-PCR were, on average, aged 24.3 years (median, 21.5 years), compared with a mean of 26.9 years (median, 26.0 years) for persons whose ulcers were not caused by *T. pallidum* (P = .03). Patients who were M-PCR positive for *T. pallidum* reported a mean duration of their ulcer of 18.2 days, compared with a mean of 14.1 days (median, 7.0 days) for all other patients (P = .088). The prevalence of syphilitic ulcers as determined by M-PCR was negatively correlated with years of schooling in a dose-response fashion: syphilitic ulcers were found in 12 (48%) of 25 patients with <6 years of schooling, 23 (42.6%) of 54 with 6 years, 15 (21.7%) of 69 with 9 years, 6 (16.7%) of 36 with 12 years, and 1 (3.3%) of 12 with >12 years of education (P = .001). The prevalence of M-PCR–negative ulcers increased with increasing years of schooling from 16% to 22.2%, 31.9%, 44.4%, and 66.7% in the respective categories (P = .001). However, there was no linear trend between education and M-PCR–defined chancroid and HSV.

Table 2. Comparison of clinical diagnosis with multiplex polymerase chain reaction–defined etiology of genital ulcers in 196 Malagasy patients.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>% sensitivity (95% CI)</th>
<th>% specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>92.9 (89.3–96.5)</td>
<td>20.1 (14.5–25.7)</td>
<td>31.9 (25.4–38.4)</td>
<td>87.5 (82.9–92.1)</td>
</tr>
<tr>
<td>Herpes</td>
<td>0.0 (98.3–99.9)</td>
<td>99.4 (98.3–99.9)</td>
<td>0.0 (28.4–41.8)</td>
<td>90.2 (86.0–94.4)</td>
</tr>
<tr>
<td>Chancroid</td>
<td>53.1 (46.1–60.1)</td>
<td>51.9 (44.9–58.9)</td>
<td>35.1 (28.4–41.8)</td>
<td>69.4 (62.9–75.9)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
When years of schooling were ignored in multivariate analysis, the probability that an ulcer was M-PCR positive for *T. pallidum* was associated with a younger age (OR per year increase, 0.94; 95% CI, 0.90–0.99; *P* = .02), ulcer detected by a clinician rather than by the patient (OR, 3.89; 95% CI, 1.40–10.78; *P* = .009), number of days since ulcer appeared (OR, 1.02; 95% CI, 1.00–1.04; *P* = .051), and no previous drug treatment for the current ulcer (OR, 2.03; 95% CI, 1.03–4.52; *P* = .042). However, when the variable “years of schooling” was added, none of the variables identified in the first model continued to contribute to the fit at the .05 significance level. In the final age-adjusted logistic regression model, the odds ratio of having a syphilitic ulcer was 0.564 (95% CI, 0.409–0.787; *P* = .0006) per 3-year increase in schooling. Adding the variables of the first model one at a time to this model did not meaningfully change the OR.

Thirteen (22.8%) of 57 women, compared with 6 (4.3%) of 139 men (OR, 6.55; 95% CI, 2.35–18.21; *P* < .001), had lesions positive by M-PCR for HSV. Detection of the ulcer by patient versus clinician was not associated with HSV infection. Of the 21 lesions reported by the clinicians as vesicular, by M-PCR 7 (33.3%) contained *T. pallidum*, 5 (23.8%) *H. ducreyi*, and 2 (9.5%) HSV. Of the 23 patients whose genital ulcers were detected by a clinician, none had lesions that were recorded as vesicles by the clinician. In multivariate analysis, gender was the only variable associated with herpetic lesions. Thirty (46.9%) of 64 patients whose ulcers contained *H. ducreyi* DNA by M-PCR used drugs previously for their current ulcer, compared with 43 (32.6%) of 132 patients who did not have chancroid (OR, 1.49; 95% CI, 1.0–2.21, *P* = .052). Negative M-PCR results were not statistically associated with prior therapy or with clinical diagnosis, although 7 (87.5%) of 8 clinical diagnoses of scabies were M-PCR negative.

Of 179 subjects, 59 (33.0%) had a reactive RPR test result, and all 59 were confirmed by TPHA. Syphilis seroreactivity was 71.7% sensitive (95% CI, 65.1–78.3) and 83.3% specific (95% CI, 77.8–88.8) compared with diagnosis by M-PCR. In persons who reported that the ulcer appeared ≥10 days earlier, sensitivity of syphilis serology relative to M-PCR was 84.4% and specificity was 82.0%; for patients whose ulcer was <10 days old, sensitivity and specificity of syphilis serology were 52.4% and 84.6%, respectively. Eight (13.3%) of 60 patients with M-PCR–negative ulcers were syphilis seroreactive, compared with 51 (42.9%) of 119 with M-PCR–positive ulcers (OR, 0.66; 95% CI, 0.54–0.79; *P* < .001).

LGV was diagnosed clinically in 2 (3.2%) of 62 patients with negative M-PCR results and in 14 (10.5%) of 133 with positive M-PCR results (*P* > .05). IgG antibodies to *C. trachomatis* were detected in 122 (78.7%) of 155 sera tested by MIF. The highest *C. trachomatis* IgG titer, 1 : 2048, was found in 1 serum sample that did not contain *C. trachomatis*–specific IgM. *T. pallidum* DNA was detected by M-PCR in the corresponding ulcer specimen. The next highest *C. trachomatis* IgG titer observed in this study population, 1 : 512, was found in 3 patients.

**Discussion**

By M-PCR, the most common causes of genital ulcers in this study in Antananarivo were *H. ducreyi* (33%) and *T. pallidum* (29%), followed by HSV (10%). Despite a high exposure rate to *C. trachomatis* observed by MIF serology, only 1 plausible case of LGV was found (IgG titer, 1 : 2048). Moreover, only 8% of the patients were clinically diagnosed with LGV. This study was thus unable to confirm the results of an earlier investigation in Antananarivo that reported LGV as the second most frequent cause of GUD [3]. The use of direct immunofluorescence to diagnose LGV in the first study may have resulted in overdiagnosis, as lesions may be contaminated with genital serovars or nonspecific fluorescence may be confused with fluorescing chlamydial particles. The use of culture to diagnose chancroid, on the other hand, may lead to underdiagnosis, given that *H. ducreyi* is a fastidious organism to grow [7].

The prevalence of genital herpes was greater than locally expected. HSV was found to be an increasingly important cause of genital ulcers in studies in sub-Saharan Africa [7–10]. Clinicians in Madagascar need to be informed about the local prevalence, clinical presentation, and management of genital herpes. Possible reasons why herpetic lesions were more common in women than in men include chance and differences in health care–seeking behaviors.

The demonstrated unreliability of clinical diagnosis, as found elsewhere [11, 12], combined with the absence of comprehensive and dependable laboratory support, argue strongly for a syndromic approach to the management of GUD in Madagascar. Based on these findings, the Malagasy STD/HIV Control Program has established national guidelines for case management that stipulate treatment of chancroid and syphilis whenever genital lesions are not confined to typical herpetic ulcers, that is vesicles or recurrent lesions. The suboptimal accuracy of syphilis serology also pleads for syndromic GUD treatment, although titers of nontreponemal screening tests can be useful for patient follow-up.

At least 1 of 4 persons in this study had taken medication that was not prescribed by a physician. National STD/HIV control activities need to foster prompt seeking of adequate STD care and discourage self-treatment and use of remedies recommended by friends or drug vendors. Educational messages need to encourage prevention, promote sexual abstinence when a genital ulcer is noticed, and facilitate treatment of sex partners. Clinicians need to examine carefully all patients with complaints of genital discharge, particularly women, for presence of ulcers.

Years of schooling, reflecting socioeconomic status, was strongly associated with primary syphilis in this study and elim-
inated in multivariate analysis all the biologically plausible variables associated with syphilitic ulcers. Syphilis disproportionately affects people at the bottom of the social ladder [13–15]. Access to affordable, patient-friendly quality care, including medications, is one tool needed to combat STDs and might be particularly critical for syphilis control. Antenatal syphilis control should be strengthened.

References