Etiologic Pattern of Genital Ulcers in Lusaka, Zambia: Has Chancroid Been Eliminated?

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Background: Genital ulcers are a public health problem in developing countries. The World Health Organization recommends the use of syndromic guidelines for sexually transmitted infection treatment in resource-constrained countries. Monitoring local etiologies provides information that may aid policy for sexually transmitted infection treatment. We investigated the etiology of genital ulcer disease among outpatients in Lusaka, Zambia.

Methodology: Swabs from genital ulcers of 200 patients were tested using polymerase chain reaction for *Treponema pallidum*, herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), *Haemophilus ducreyi*, and *Chlamydia trachomatis.*

Results: The prevalence of the detected pathogens was as follows; HSV-2, 28%; *T. pallidum*, 11.5%; *C. trachomatis*, 3%; HSV-1, 0.5%; and *H. ducreyi*, 0%. Coinfection with HSV-2 and *T. pallidum* was 1.5%, and coinfection of HSV-2 and *C. trachomatis* was 1%. In 55% of the patients, no etiologic diagnosis could be established.

Conclusions: *H. ducreyi* was not detected, whereas HSV-2 and *T. pallidum* were the commonest pathogens. Nondetection of *H. ducreyi* requires further studies. If the present findings are validated, treatment guidelines would require to be revised in Zambia.

Sexually transmitted infections (STIs) remain a major public health problem in developing countries. Genital ulceration is common in these countries and has been associated with increased risk of human immunodeficiency virus (HIV) transmission.[1] The World Health Organization (WHO) recommends the use of syndromic management of STIs in resource-constrained settings, which is based on groups of consistent symptoms and easily recognizable clinical findings, to arrive at a diagnosis.[2] The recommended treatment according to WHO should be based on the local etiologies and microbial sensitivity patterns, whereas treatment of genital herpes is recommended when the seroprevalence of herpes simplex virus type 2 (HSV-2) is greater than 30%.[3] The syndromic approach is cost-effective, and treatment covers for all common causes and coinfections, which otherwise could be missed. Analyzing etiologic causes provides necessary information for ensuring that the treatment guidelines are in line with the current disease patterns. To our knowledge, there are no recent studies on genital ulcer disease (GUD) etiology in Zambia. A study conducted at an STI clinic in Lusaka in the 1990s compiled clinical diagnoses of genital ulcers and found that chancroid was the commonest diagnosis in men (47%), whereas syphilis was commonest among women (39%).[4] The present study sought to document the microbiologic etiology of genital ulcers among patients presenting with genital ulcers attending primary health care clinics in Lusaka district. We used molecular diagnostic methods to ascertain the etiology of the ulcers as opposed to clinical diagnoses that have been used previously in this setting.

PATIENTS AND METHODS

Patients

Of a total of 26 public health centers in Lusaka, 10 were selected for the study. Based on data from the District Health Information System, health centers with the highest incidence of STIs were selected. Consecutive patients aged 16 years or older reporting genital ulcers at the outpatient departments were recruited between May 3 and June 4, 2010. Patients younger than 16 years, the legal age of consent in Zambia, and those who reported having taken antibiotics 2 weeks before the study were excluded. Ten nurse midwives who were trained as research assistants administered the questionnaire in English, Nyanja, or Bemba. Translation and back translation of the questionnaire were done by independent professional translators. The questionnaire included questions on demographics and sexual behavior of the patients. A physical examination of the genitalia was performed, including a speculum examination in the women. A swab of the genital ulcer was taken from all patients. Before the actual data collection, a pilot study was conducted with 15 patients with genital ulceration being interviewed and examined to pretest the data collection tools, and specimens were collected for trial runs in the laboratory. An oral mucosal transudate HIV antibody test was performed on consenting patients, using Oraquick rapid HIV 1/2 antibody test. Those who did not consent to the HIV test were still eligible for the study.

Specimen Collection and Storage

COPAN Venturi Transystem swabs (Copan Italia, Bresia, Italy) were used to collect specimens, which were transferred to the laboratory at room temperature within 3 hours of collection. Specimen testing and storage were done at the Kaposi's Sarcoma research laboratory at the university teaching hospital in Lusaka, Zambia. On the same day, each swab was transferred in a 2 mL cryovial containing 400 μL of phosphate-buffered saline and stored at −80°C until DNA extraction. DNA was extracted using the QIAmp DNA mini kit from QIAGEN (www.qiagen.com).

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The authors thank the patients who participated in the study. The authors also thank David Rutagwera for coordinating the laboratory work with Chisanga Mulombwa's assistance, the research assistants, and the management and staff at the KS research Laboratory at the University Teaching Hospital in Lusaka. The authors are also grateful to Tania Crucitti for reviewing the article and for the valuable comments.

Supported by the University of Bergen, Bergen, Norway.

The authors declare no conflicts of interest.

Design and plan of the study were contributed by M.M. and I.F.S. Fieldwork was conducted by M.M. Analysis, write up, and approval of the final version were all provided by M.M., I.F.S., and A.B.

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Received for publication February 7, 2012, and accepted July 20, 2012.
DOI: 10.1097/OLQ.0b013e31826ae97d

Six aliquots of 15 μL of the extract were made and stored at −20°C until testing. Five were used for polymerase chain reaction (PCR) and 1 was used for backup/long-term storage at −20°C. Primerdesign kits (www.primerdesign.co.uk) were used for the amplification and detection of the pathogens of interest. We tested for *Treponema pallidum*, *Chlamydia trachomatis*, herpes simplex virus types 1 and 2 (HSV-1, HSV-2) and *Haemophilus ducreyi*. We checked for *C. trachomatis* to detect possible lymphogranuloma venereum (LGV) infection; however the kit used did not distinguish between different serovars, thus making it impossible to identify cases of LGV. Five real-time qualitative PCRs were performed on each extracted DNA specimen, each using a set of 2 primers and a probe to target 1 of the 5 organisms, *T. pallidum*, *H. ducreyi*, *C. trachomatis*, HSV-1, and HSV-2 using specific kits for each organism predesigned and prevalidated by PrimerDesign Ltd. (Southampton, United Kingdom; www.primerdesign.co.uk). To check the quality of the DNA extract, primers/probes targeting the actin β gene were run with the first PCR for each set of 5 PCRs. To validate each run, 6 standards, positive, and negative controls were run. An internal extraction control was also run with each sample to rule out PCR inhibition in each PCR reaction well. Data collection was performed using both FAM and HEX channels of the Strategene Mx3000p real-time platform.

STATISTICAL ANALYSIS AND ETHICAL ISSUES

Analyses were performed using the SPSS (PASW Statistics 18; IBM SPSS Statistics, Chicago, IL). χ^2 tests were done to test for associations. A P value less than 0.05 was considered significant. Logistic regression with odds ratios (ORs) and 95% confidence intervals (CIs) was used to test the associations between signs/symptoms and HSV-2 versus T. pallidum versus no pathogen detection.

Ethical clearance was obtained from the research and ethics committees of the University of Zambia and Western Norway. The Ministry of Health in Zambia granted the permission to conduct the study at the health centers under the jurisdiction of Lusaka District Health authorities. Written informed consent was obtained from all patients, before participation, and separate written consent was obtained for saliva-based HIV testing. We selected a saliva-based test instead of a blood test because it is less invasive and was likely to give less refusals. However, the Ministry of Health's policy recognizes saliva-based HIV testing for research purposes only, whereas serum, plasma, or whole blood is required for routine testing. The patients were thus thoroughly informed that the result would only be available for research purposes. All patients were treated according to the national treatment guidelines for STIs and counseled for HIV by a clinician from the facility. Patients who were willing to know their HIV status were referred for HIV testing within the health facilities. The research assistants also actively encouraged those who showed willingness to know their status to go to the voluntary counseling and testing room.

The PCR results were sent to the respective clinics, and patients were invited back for their results. Unfortunately, it took 8 months before the results could be ready because the laboratory work took longer than expected.

RESULTS

A total of 205 specimens from the same number of patients were collected. Of these, 5 specimens had 1 to 2 invalid readings on the 5 runs, and these were excluded. Only the set of results for the 200 patients with valid PCR readings for all 5 pathogens was included in the analysis. Of these, 100 were males, and 100 were females. No refusals were recorded for participation. However, only 128 (64%) of the patients consented to be tested for HIV. The mean age was 28 years, and more than half of the patients had secondary or tertiary school education. Most of the patients had multiple (64%), painful (73%), nonbleeding ulcers (81%), and no genital discharge (77%), and most patients reported that episode as their first occurrence of the ulcer (70%). Only 18% of the patients had palpable bilateral inguinal nodes (Table 1).

H. ducreyi was not detected in any of the specimens. The commonest pathogen was HSV-2 followed by *T. Pallidum*. *C. trachomatis* prevalence was low, whereas HSV-1 was negligible. Fifty-five percent of the patients did not have any pathogens detected from their ulcers (Table 2). Compared with those without pathogens detected, patients with *T. pallidum* had a significantly higher likelihood of having 3 ulcers (OR, 2.7; 95% CI, 1.02–7.06), whereas those with HSV-2 were less likely, although not significant, to have multiple ulcers (OR, 0.8; 95% CI, 0.45–1.61). Among the patients who reported duration of ulcers lasting for more than 2 weeks, 68% did not have pathogens detected, and of those who reported past ulcers more than once, more than half had no pathogens detected (56%). Most of the patients (68%) who had HSV-2 tested positive for HIV. Patients with no pathogens being detected were less likely to test positive for HIV than were for other patients (OR, 0.3; 95% CI, 0.1–0.7). Although not significant, female patients with *T. pallidum* were less likely to have intravaginal or cervical ulcers compared with those with HSV-2, whereas patients with no pathogens detected in their ulcers had a higher likelihood (*T. pallidum*: OR, 0.40; 95% CI, 0.07–2.3; no pathogen ulcers: OR, 1.8; 95% CI, 0.7–4.5).

DISCUSSION

The commonest cause of genital ulcers detected by PCR in this study population was HSV-2, and this is consistent with other studies from Zambia and other African countries.[5–8] *T. pallidum* was the second most common, whereas *C. trachomatis* was detected in only a small proportion of the patients. *H. ducreyi* was not detected in any of the patients. The zero prevalence reported in this study could indicate a decline in chancroid prevalence to negligible levels or perhaps elimination of the disease. Reports of declines in chancroid and other bacterial STIs have been documented in the region, and some of the factors possibly explaining this are successful antibiotic treatment through the use of the syndromic approach to treat STIs, combined with reduction in sexual risk behavior.[9,10] Zambia has had an STI control program since 1980, and adapted WHO syndromic guidelines have been in use since 1990.[4,11] As has been suggested in Botswana, syndromic management and the use of antibiotics could possibly have played a role in the reduction of chancroid in this setting. In addition, because chancroid usually presents as an overt and very painful disease, patients are likely to seek treatment. The feasibility of chancroid eradication has been well documented because its short infectivity period showing its vulnerability and the fact that it requires a high partner change rate make it less of a problem in the general population. Chancroid tends to concentrate among persons with a high partner turnover such as sex workers. In addition, it has a single, human reservoir, and single-dose drugs effective against *H. ducreyi* are readily available and cheap, and even simple interventions such as washing with water and soap have been shown to reduce transmission.[12]

A high HIV prevalence was observed among the patients with GUD (49%). The HIV seroprevalence was higher among patients with HSV-2 infection (68%).Systematic reviews of epidemiologic data have shown HSV-2 infection as being a significant risk factor for HIV acquisition,[13,14] and particularly

TABLE 1. Comparison of Patient Characteristics, Symptoms, and Findings for Different PCR Results

	Total,* %	HSV-2, n (%)	*T. pallidum*, n (%)	Ulcers Without Pathogens Detected, n (%)	*P*
Age, y					0.365
16–20	27	8 (30.8)	2 (7.7)	16 (61.5)	
21–24	45	12 (26.7)	9 (20.0)	24 (53.3)	
25–29	50	14 (28.0)	5 (10.0)	31 (62.0)	
30–34	41	14 (35.0)	6 (15.0)	20 (50.0)	
35+	37	9 (25.0)	1 (2.8)	26 (72.2)	
Sex					0.536
Male	100	27 (27.3)	14 (14.1)	58 (58.6)	
Female	100	30 (30.6)	9 (9.2)	59 (60.2)	
Duration of ulcer					**0.035**
1–6 d	61	19 (31.7)	4 (6.7)	37 (61.7)	
1–2 wk	55	22 (40.7)	8 (14.8)	24 (44.4)	
>2 wk	84	16 (19.3)	11 (13.3)	56 (67.5)	
No. times experienced genital ulcers in the past					**0.020**
Never	125	28 (2.0)	19 (15.6)	75 (61.5)	
Once	17	9 (52.9)	0 (0)	8 (47.1)	
More than once	37	15 (40.5)	1 (2.7)	21 (56.8)	
Reported bleeding					0.323
Yes	38	9 (23.7)	7 (18.4)	22 (57.9)	
No	160	48 (30.6)	16 (10.2)	93 (59.2)	
Reported painful ulcers					0.545
Yes	145	41 (28.5)	15 (10.4)	88 (61.1)	
No	54	16 (30.8)	8 (15.4)	28 (53.8)	
Reported genital discharge					0.460
Present	43	15 (35.7)	3 (7.1)	24 (57.1)	
Absent	144	41 (28.9)	19 (13.4)	82 (57.7)	
Observed no. ulcers					0.100
1	71	26 (37.1)	4 (5.7)	40 (57.1)	
2	37	8 (22.2)	3 (8.3)	25 (69.4)	
3	91	23 (25.6)	15 (16.7)	52 (57.8)	
Observed shape of ulcer					0.330
Round	79	21 (26.6)	5 (6.3)	53 (67.1)	
Oval	37	12 (33.3)	4 (11.1)	20 (55.6)	
Irregular/vesicular	91	22 (28.9)	12 (15.8)	42 (55.3)	
Palpable inguinal nodes					0.858
Absent	147	43 (29.7)	17 (11.7)	85 (58.6)	
Present unilaterally	17	4 (25.0)	3 (18.8)	9 (56.3)	
Present bilaterally	36	10 (27.8)	3 (8.3)	23 (63.9)	
Speculum examination					0.282
No ulcers present	51	17 (34.7)	7 (14.3)	25 (51.0)	
Present on vaginal wall	36	10 (27.8)	2 (5.6)	24 (66.7)	
Present around the cervix	10	2 (20.0)	0 (0.0)	8 (80.0)	
Results of HIV test					**0.004**
Positive	63	26 (41.9)	8 (12.9)	28 (45.2)	
Negative	65	11 (17.2)	6 (9.4)	47 (73.4)	

Significant figures are in bold.

*Patients with *C. trachomatis* and HSV-1 included.

incident HSV-2 infection has been shown to yield a higher risk compared with already existing herpes infection.[15] Furthermore, people who are infected with both experience more severe genital herpes infection and have frequent recurrences and prolonged HSV-2 shedding.[16]

This study showed a high proportion of ulcers where no pathogens were detected (55%). Wawer et al.[17] also found negative PCR results for 51% of patients that had presented with genital ulcers in a study in Uganda, whereas a recent study in South Africa reported a lower estimate (18%).[18] The reasons for

TABLE 2. Etiology of Genital Ulcers

Results of PCR	Total, n (%)	Men, n (%)	Women, n (%)
HSV-2	56 (28.0)	26 (26.0)	30 (30.0)
HSV-1	1 (0.5)	0 (0)	1 (1.0)
T. pallidum	23 (11.5)	14 (14.0)	9 (9.0)
C. trachomatis	6 (3.0)	2 (2.0)	4 (4.0)
HSV-2 and *T. pallidum*	2 (1.0)	1 (1.0)	1 (1.0)
HSV-2 and *C. trachomatis*	2 (1.0)	1 (1.0)	1 (1.0)
Ulcers with no pathogens detected	110 (55.0)	56 (56.0)	54 (54.0)

this high proportion of negative PCR results could include poor specimen collection and storage, alternative etiologies that were not tested, and traumatic abrasions and excoriations. The former, however, is unlikely because only specimens that tested positive for actin β gene, the DNA internal extraction control, were included in the analysis, giving an assurance of good quality of the collected material. Moreover, PCR testing techniques are more sensitive than are culture and are less subject to collection and transport problems.[19] We found a strong association between having no pathogen detected and old ulcers. Some authors also found an association between shorter duration of the ulcer and higher PCR detection of HSV-2 and postulated that the longer the duration of the ulcer, the less is the likelihood of identifying the HSV-2.[8] Wald et al.[20] in a prospective cohort of women seropositive HSV-2 were able to detect HSV-2 by PCR only in approximately a third of the follow-up period even if they clinically had genital herpes. They also found that shedding of HSV-2 was clustered in days or episodes. In our study, both patients with HSV-2 infection and those with no pathogens detected were more likely to report past ulcers compared with those with syphilis. Recurrent episodes are a classical phenomenon of genital herpes. We believe that many of the patients in whom we did not detect any pathogens may have had HSV-2. Although patients that reported having taken antibiotics in the past 2 weeks before the study were excluded, this does not guarantee that none of the patients had taken any medication that could have affected pathogen detection because antibiotic use may have been underreported. Among other documented but very rare organisms that could cause ulcers are *Klebsiella granulomatis*, a bacterium that causes donovanosis, and Epstein Barr virus, cytomegalovirus, *Gardnerella vaginalis*, Behçets disease, *Staphylococcus aureus*, and idiopathic genital ulceration associated with HIV infection.[21–23] Donovanosis was shown in the 1970s as being endemic in Zambia, Zimbabwe, and South Africa.[24,25] However studies have found all these organisms to be uncommon; thus, we did not test for them.[26] Although *S. aureus* has been found in genital ulcers, it could be a result of superadded infection of the wound rather than the actual cause.

Only 8 (3%) of the patients had *C. trachomatis* detected from the ulcers. It was noted that 6 of the 8 patients reported genital discharge. Because we are unable to distinguish the different serovars, which made it impossible to identify cases of LGV, we were unable to tell whether chlamydia could have been the primary cause or was due to contamination of the ulcer.

Our study could have been subject to selection bias in that not all patients with GUD seek care and those with mild disease or fewer resources are less likely to seek treatment. That no refusals for participation were recorded could possibly indicate that patients found it difficult to refuse. A contrasting interpretation would be that the high study participation reflected that patients felt assured of confidentiality and that obtaining a PCR result could have been an incentive for participating. We however acknowledge as a weakness of the long turnaround time before the results were ready and the nonprovision of HIV test results to patients. These are areas for improvement in future research. Recall bias could have been another possible source of bias because certain questions were based on history. Patients with more severe symptoms and who sought care are likely to report more information than those with mild symptoms. Certain questions may have been perceived to be intrusive, and this may have led to underreporting of previous ulcers too.

That more sensitive molecular diagnostic methods were used in this study and considering that patients with chancroid are very likely to seek symptomatic relief make us believe that the prevalence of chancroid could be negligible in Lusaka. However, because of the small sample, these findings should ideally be validated by conducting similar studies in multiple sites. Etiologic surveillance, although expensive, provides necessary information to guide treatment policy and should be considered. The success of syndromic management of STIs is dependant upon local up-to-date information of the pathogens causing the syndromes. This study provides a basis for further research. The findings indicate that STI treatment guidelines need to be revised. Currently the practice is that, a patient with GUD is treated for syphilis, chancroid and LGV, with 3 doses of intramuscular benzathine penicillin 2.4 MU given weekly, ciprofloxacin 500mg twice daily for 3 days and doxycyclin 100mg twice daily for 14 days.[27] Treatment for chancroid would need to be removed from the GUD algorithm. Instead, patients with GUD should be given benzathine penicillin and acyclovir, considering the high HSV-2 prevalence in and the burden of HIV. Suppressive therapy is the best option for HSV-2 treatment in this setting; however, the cost is prohibitive.[28] Episodic treatment is more feasible and affordable[5] than to do nothing. Training patients with HSV-2 on symptom recognition and prompt care seeking would be of paramount importance to yield the best results. Operationalizing this also entails adding acyclovir to the essential drug list, ensuring constant supplies in the health facilities, and monitoring for drug resistance.[28,29]

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