Etiological Surveillance of Genital Ulcer Syndrome in South Africa: 2019 to 2020

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Background: Herpes simplex virus (HSV) has been the leading cause of genital ulcer syndrome (GUS) in South Africa for more than a decade, and acyclovir therapy is incorporated into syndromic management guidelines. We conducted surveillance at 3 sentinel sites to define the common sexually transmitted etiologies of GUS and to determine whether current syndromic management is appropriate. Secondary objectives of surveillance were to determine the seroprevalence of coinfections (HIV, syphilis, HSV-2) in persons presenting with GUS.

Methods: Consecutive, consenting adult men and women presenting with visible genital ulceration were enrolled between January 1, 2019, and December 31, 2020. Genital ulcer swab and blood specimens were collected and transported to a central sexually transmitted infection reference laboratory in Johannesburg.

Results: Among 190 participants with GUS, HSV-2 was the most frequently detected ulcer pathogen (49.0%; 95% confidence interval [CI], 41.9%–56.1%). The relative prevalence of the second most common ulcer-derived pathogen, *Treponema pallidum,* was 26.3% (95% CI, 20.5%–33.1%), with 90% of primary syphilis cases having a positive rapid plasma reagin (RPR) titer. Male sex was independently associated with primary syphilis compared with herpetic ulcers, after adjusting for the effect of casual sex partners and other exposures (adjusted odds ratio, 3.53; 95% CI, 1.35–9.21; $P = 0.010$). The overall HIV prevalence among participants was 41.3% (78 of 189; 95% CI, 34.2%–48.6%).

Conclusions: Herpes simplex virus 2 remains the predominant cause of GUS, justifying the continued use of acyclovir in syndromic guidelines. Adequate supplies of benzathine penicillin G for syphilis treatment are essential at primary health care level, in addition to the provision of syphilis and HIV risk reduction services.

Clinical surveillance of sexually transmitted infection (STI) syndromes at sentinel sites in South Africa has revealed that genital ulcer syndrome (GUS) is among the most common STI clinical manifestations.[1] Approximately 4500 GUS cases were reported in adult males and females presenting to approximately 270 primary health care centers (PHCs) serving as sentinel clinical surveillance sites between April 2019 and March 2020 (National Department of Health, District Health Information System). With the onset and establishment of the HIV epidemic in South Africa in the early 2000s, herpes simplex virus (HSV; specifically HSV type 2 [HSV-2]) emerged and has remained the leading cause of GUS.[2] Before this, a bacterial cause, *Haemophilus ducreyi*, predominated.[3] However, since the addition of erythromycin to GUS national treatment algorithms in the late 1990s, chancroid has not been identified in samples from sentinel surveillance studies in recent years.[2]

The national syndromic management guidelines for GUS have mirrored the changing epidemiology of GUS in the country. In 2008, acyclovir was included in the treatment algorithm for the treatment of HSV in HIV-infected persons or in those whose HIV status was unknown.[4] *Treponema pallidum* is the second most common ulcer-derived STI pathogen, and benzathine penicillin G (BPG) is included as treatment in persons who self-report being sexually active in the preceding 3-month period. A single dose of azithromycin, for chancroid, is considered only in the event of nonhealing following therapy for HSV and syphilis. Lymphogranuloma venereum (LGV) caused by *Chlamydia trachomatis* genovars L1–L3 is only sporadically detected in genital ulcer surveillance studies.[2] A separate management algorithm for bubo, which includes once-weekly dosing of azithromycin for 3 weeks, covers the 2 most commonly implicated agents of this condition—chancroid and LGV.[4] Although granuloma inguinale (donovanosis) accounted for a significant proportion of genital ulceration seen in KwaZulu Natal province in the latter part of the 20th century, this condition has been virtually eliminated and has not been reported in recent years.[5]

The Centre for HIV & STI at the National Institute for Communicable Diseases (NICD) has been conducting STI etiological surveillance since 2007 to revise and update national syndromic management guidelines in use at PHCs throughout the country. From 2019 to 2020, surveillance was conducted at 3 sentinel sites in 3 provinces: Gauteng, Western Cape, and KwaZulu Natal (KZN). We describe the results of STI surveillance in adult males and females presenting with visible genital ulceration to the 3 facilities in 2019 to 2020. The primary objective of surveillance was to determine the STI etiologies of GUS. Secondary objectives were to determine the seroprevalence of STI coinfections (i.e., HIV, syphilis, HSV-2) among participants presenting with genital ulceration.

METHODS

Design

We conducted an observational cross-sectional study of the prevalence of STI etiologies.

Setting

Three PHCs that are high-volume STI sentinel sites were as follows: Alexandra Health Centre (Gauteng), Spencer Road Clinic

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(Western Cape), and Prince Cyril Zulu Communicable Disease Centre (KZN). Surveillance has been conducted annually at Alexandra Health Centre since 2008. Surveillance was commenced at Spencer Road Clinic in May 2019 and at Prince Cyril Zulu CDC in April 2020.

Participants

Consecutive consenting adult males and females 18 years or older presenting with clinically confirmed genital ulceration to the selected PHCs, between January 1, 2019, and December 31, 2020, were enrolled. The target sample size per site per year was 100 cases of GUS. For an HSV prevalence of 65% with a 95% confidence level and a precision of 10%, it was estimated that 87 participants would need to be recruited. A nurse-administered questionnaire (paper-based or electronic) was used to document demographic and clinical information. Participant information was anonymized by using unique survey ID numbers for each.

Specimen Collection

Surveillance nurses collected Dacron swab specimens of material from the base and edges of ulcerative lesions. Each swab was rolled over the center of a labeled glass slide, which was allowed to air dry. The swab then was placed in a sterile container. In addition, a 10-mL venous blood specimen was collected from each participant for serological testing. All slides, swabs, and blood specimens were transported on ice to the STI reference laboratory at the NICD in Johannesburg.

Laboratory Testing

Molecular Testing

DNA was extracted from swab specimens using 1 of 2 automated DNA extractors, that is, MagNA Pure Compact system (Roche, Basel, Switzerland) or QIAcube HT instrument (Qiagen, Hilden, Germany). Specimen extracts were tested using a validated in-house multiplex real-time (RT) polymerase chain reaction (PCR) assay for ulcer-causing STI pathogens: HSV, *T. pallidum*, *H. ducreyi* and *C. trachomatis*.[6] The multiplex RT-PCR contains an RNAse P human DNA target (internal control) to control for DNA extraction efficiency. All samples with a negative internal control result were reextracted and retested.

Herpes simplex virus–positive DNA extracts were subtyped into HSV-1 and HSV-2 using a commercial type–specific RT-PCR (Sacace Biotechnologies, Como, Italy). If *C. trachomatis* was detected, an in-house simplex RT-PCR was used to determine whether it belonged to a serovar associated with LGV (*C. trachomatis* L1–3). All RT-PCR assays were performed on the RotorGene Q PCR platform (Qiagen).

Microscopy

Glass slides were Giemsa stained and examined for the presence of intracellular Donovan bodies within monocytes and macrophages for the diagnosis of granuloma inguinale or donovanosis caused by *Klebsiella granulomatis*.

Serology

Serum specimens were screened for HIV using the Architect HIV Ag/Ab combo kit (Abbott, Wiesbaden, Germany) and for HSV-2 infection using the Focus HerpeSelect 2 ELISA IgG assay (Focus Diagnostics, Cypress, CA). Syphilis coinfection was detected by screening with an automated specific treponemal assay (Architect Syphilis TP; Abbott Diagnostics) and reflexed, if positive, to a manual rapid plasma reagin (RPR) Immutrep RPR assay (Omega Diagnostics Ltd, Alva, United Kingdom).

Data Analysis

Data from clinical questionnaires and Excel spreadsheets of laboratory data were exported into Stata 16 (Stata Corporation, College Station, TX) for analysis. The enrolled participants were described using frequencies and proportions for categorical data, and medians and interquartile ranges (IQRs) for continuous variables. The relative prevalence of the different STI pathogens and seroprevalence of HIV, HSV-2, and RPR were determined as proportions with 95% confidence intervals (CIs) around them. Pearson χ^2 and Fisher exact tests were used to test for associations between various exposure variables and ulcer pathogens, with the level of significance set at $P < 0.05$. Multivariable logistic regression analysis with stepwise backward elimination was done to determine the factors independently associated with *T. pallidum* as opposed to HSV ulceration. Exposure variables with the largest P value were excluded at each iteration of the model, until we had 1 variable for every 10 outcomes of *T. pallidum* ulcers. Model prediction and comparison of area under the curve of associated receiver operating curves was used to determine if the logistic regression model with an interaction between sex and having a casual sex partner fit the data better than one without.

RESULTS

Participant Demographic, Behavioral, and Clinical Characteristics

Among 190 participants with GUS enrolled at the 3 sites, genital ulceration was the primary complaint in the majority (171 [90%]). Genital ulcer syndrome was the secondary syndrome in 19 participants who presented with male urethritis (n = 7) and vaginal discharge (n = 12). In total, 126 (66.3%) were male (Table 1). The median age of participants was 32 years (IQR, 26–39 years), and less than one-fifth (16.8%) were younger than 25 years. The majority self-reported heterosexual orientation, and nearly 30% reported recent sex with a casual (nonregular) partner. Approximately 20% of

TABLE 1. Demographic and Behavioral Characteristics of Participants With GUS (N = 190)

Variable	n (%)
Male	126 (66.3)
Black Africans	188 (98.9)
Current age (n = 189), median (IQR), y	32 (26–39)
Age <25 y (n = 189)	32 (16.8)
Age at first sex, median (IQR), y	17 (16–19)
Heterosexual orientation	186 (97.9)
Condom use at most recent sexual encounter	25 (13.2)
Sexual encounter with a nonregular sexual partner (past 3 mo)	56 (29.5)
Median (range) no. partners (past 3 mo)	1 (0–8)
≥2 sexual partners (past 3 mo)	52 (27.4)
Sex outside province (past 3 mo)	42 (22.1)
Sex outside the country (past 3 mo)	34 (17.9)
STI syndrome diagnosed in the past 12 mo	39 (20.5)
Presenting with ≥2 syndromes	33 (17.4)
Know their HIV status	160 (84.2)
Self-reported being HIV positive*	47 (29.4)
Taken ARVs in the past 3 d†	36 (76.6)
Males ever circumcised (n = 126)	51 (40.5)
Males ever circumcised medically‡	26 (49.0)

*Denominator = individuals who know HIV status (n = 160).
†Denominator = individuals who self-reported being HIV positive (n = 47).
‡Denominator = males who were circumcised (n = 51).
ARVs indicates antiretroviral therapy.

participants overall had been diagnosed with an STI syndrome within the preceding 12-month period, and 17% presented with clinical manifestations of 2 or more STI syndromes. Of the 30% of participants who stated they were HIV infected, only 77% had taken antiretroviral drugs within the last 3 days. Overall, 51 of 126 men (40.5%) were circumcised, of these 25 of 51 (49%) had been medically circumcised. Men presenting with GUS were older than their female counterparts: median ages of males and females were 34 years (IQR, 27–42 years) and 28 years (IQR, 23–33 years), respectively. Only 11.1% (14 of 126) of men were younger than 25 years compared with 28.1% (18 of 64) of women. When sexual behavioral risks were stratified by sex, men were more likely than women to report having casual sex partners (39.7% vs. 9.4%, $P < 0.001$) and multiple partners (38.1% vs. 6.2%, $P < 0.001$).

Laboratory Results

STI Etiologies of GUS

Among 190 GUS cases (Fig. 1), the major PCR-detectable cause was HSV in 49.0% (93; 95% CI, 41.9%–56.1%), followed by *T. pallidum* in 26.3% (50; 95% CI, 20.2%–33.1%). Type-specific PCR revealed that all HSV-associated ulcerations were caused by HSV-2. Most cases of genital herpes were also HSV-2 seropositive and represented reactivation disease (75 of 90 [83.3%]; 95% CI, 74.0%–89.8%), as opposed to first-episode HSV-2 ulceration. Most pathogen-detectable GUS cases had a single etiology (136 of 190 [71.6%]). Only 4 participants (2.1%) had mixed etiology with HSV and *T. pallidum* coinfection. Lymphogranuloma venereum was detected in only 1 male participant at the KZN facility. There were no cases of chancroid or donovanosis. An ulcer-derived pathogen was not identified in approximately 26% GUS cases (50; 95% CI, 20.5%–33.1%). Statistical analysis to reliably detect significant etiological differences by site was limited by small sample sizes for GUS.

Further analysis was conducted to discern demographic, behavioral, and clinical associations with the 2 most common sexually transmitted causative agents: genital herpes infection versus primary syphilis (n = 135). Crude unadjusted analysis revealed that *T. pallidum* ulceration was associated with male sex and having a casual partner in the previous 3 months (Table 2). In the final multivariable logistic regression model, there was evidence of association between male sex and primary syphilis, after controlling for the effect of casual sex partners, and history of recent sex in a different province or a different country (adjusted odds ratio [OR], 3.53; 95% CI, 1.35–9.21; $P = 0.010$; Table 2). There was no difference in the area under the curve between the model with the interaction term and that without ($P = 0.663$), suggesting lack of effect modification by casual sex partners.

A majority of primary syphilis cases (90% [45 of 50]) were RPR seropositive, with 77.8% having RPR titers ≥8 (modal RPR, 32; range, 1–128). The overall HIV seropositivity in GUS was 41.3% (78 of 189; 95% CI, 34.2%–48.6%). There was no association between PCR-detected ulcer etiology and HIV status of participants. Those who were seropositive for HSV-2 were 2.39 times more likely to be HIV positive (95% CI, 1.13–5.06; $P = 0.019$). Participants without detectable ulcer pathogens were 56% less likely to be HSV-2 seropositive (OR, 0.44; 95% CI, 0.21–0.90; $P = 0.021$) and 88% less likely to be RPR positive (OR, 0.12; 95% CI, 0.03–0.40), compared with those in whom STI pathogens were identified.

DISCUSSION

Our findings reveal that HSV-2 remains the predominant cause of genital ulceration in our population, and therefore, the continued incorporation of acyclovir into GUS treatment algorithms is appropriate.[7] The prevalence of HSV was lower during the 2019–2020 surveillance period, relative to previous years: Johannesburg GUS surveillance conducted between 2007 and 2015 revealed an overall HSV prevalence of 60.7% (which ranged from approximately 55% to 75% for each year of surveillance).[2] This reduction may be attributed to an improvement in HIV screening and treatment coverage in the country[8] with a resultant decline in the relative prevalence of HSV-reactivation disease, as well as to an increased uptake of male medical circumcision services, which may lead to a reduction in incident HSV.[9] We did not detect HSV-1 as a cause of genital ulceration, unlike in Western Europe and North America, where it is an important cause of first-episode genital herpes in the reproductive age group.[10] This has been attributed to a higher standard of living in high-income countries with a reduction in childhood exposure via oral transmission and acquisition of infection in early adulthood via orogenital sexual transmission. In contrast, HSV-2 accounts for the major proportion of genital herpes seen in Asia and Africa.[2,11]

A notable and concerning finding is the increase in the relative prevalence of ulcer-derived *T. pallidum*. Historically, the relative prevalence of *T. pallidum* in genital ulcer disease has been approximately 10% or less over the years, although it was the second most common ulcer-derived pathogen.[2] In keeping with a global shortage of the active pharmaceutical ingredient, as well as local regulatory issues around registration of BPG, there have been stock-outs of the drug at public sector facilities in the country.[12] This may also account for an increase in congenital syphilis clinical notifications reported via the NICD Notifiable Medical Conditions surveillance platform since July 2017.[13] These results mirror an increase in RPR-positive results from infants and children younger than 24 months in the public health sector, which suggests increased antenatal exposure to syphilis.[14] A national antenatal syphilis serosurvey conducted in 2019 corroborates these findings:

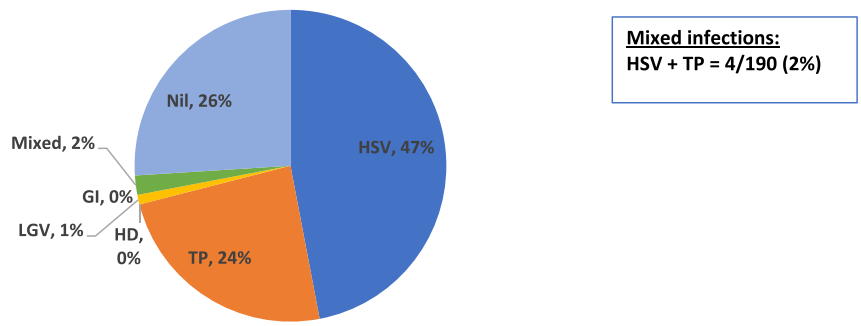

Figure 1. Relative prevalence of STI pathogens in GUS (N = 190). GI, granuloma inguinale; HD, *Haemophilus ducreyi*; TP, *Treponema pallidum*. Figure 1 can be viewed online in color at www.stdjournal.com.

TABLE 2. Comparison of Demographic Characteristics in Participants With Genital Herpes and Primary Syphilis (n = 135)

Demographic Characteristics	HSV Only* (n = 89), n (%; 95% CI)	TP Only† (n = 46), n (%; 95% CI)	Univariable (TP Only vs. HSV Only), OR (95% CI)	*P*	Multivariable (TP Only vs. HSV Only), OR (95% CI)	*P*
Male sex	52 (58.4; 47.5–68.8)	39 (84.8; 71.1–93.7)	3.96 (1.60–9.83)	0.002	3.53 (1.35–9.21)	0.010
Age <25 y	16 (18.0; 10.6–27.5)	6 (13.0; 4.9–26.3)	0.68 (0.25–1.89)	0.462‡		
Black African	89 (100.0; 95.9–100)	44 (95.7; 85.2–99.5)	—	0.114‡		
Heterosexual orientation	87 (97.8; 92.1–99.7)	44 (95.7; 85.2–99.5)	0.51 (0.07–3.71)	0.605‡		
Condom use at last sex	15 (16.9; 9.8–26.3)	3 (6.5; 1.4–17.9)	0.34 (0.09–1.26)	0.114‡		
Nonregular sex partner (past 3 mo)	20 (22.5; 14.3–32.6)	20 (43.5; 28.9–58.9)	2.65 (1.23–5.71)	0.011	2.23 (0.93–5.30)	0.071
≥2 sexual partners (past 3 mo)	19 (21.3; 13.4–31.3)	16 (34.8; 21.4–50.2)	1.96 (0.89–4.33)	0.091		
Sex outside province (past 3 mo)	21 (23.6; 15.2–33.8)	6 (13.0; 4.9–26.3)	0.49 (0.18–1.30)	0.146	0.37 (0.12–1.10)	0.073
Sex outside country (past 3 mo)	20 (22.5; 14.3–32.6)	5 (10.9; 3.6–23.6)	0.42 (0.15–1.21)	0.109‡	0.41 (0.14–1.24)	0.114
STI syndrome diagnosed in past 12 mo	19 (21.4; 13.4–31.3)	8 (17.4; 7.8–31.4)	0.78 (0.31–1.94)	0.586		
Circumcised§	22 (42.3; 28.7–56.8)	15 (38.5; 23.4–55.4)	0.85 (0.37–1.99)	0.712		
HIV seropositive	39/88 (44.3; 33.7–55.3)	19 (41.3; 27.0–56.8)	0.88 (0.43–1.82)	0.738		

*HSV: ulceration caused by herpes simplex virus.

†TP: ulceration caused by *T. pallidum.*

‡Fisher exact test.

§Denominators are males (n = 52 and n = 39) for HSV only and TP only, respectively.

maternal RPR seroprevalence increased by 30% from approximately 2% (95% CI, 1.8%–2.2%) in 2015 to 2.6% (95% CI, 2.4%–2.9%) in 2019.[15] Prescriptions of BPG require health care workers to complete extensive applications for the use of unregistered medication, and our surveillance site in KZN did not stock supplies of BPG for STI syndromic treatment. The current national guidelines account for BPG shortages by recommending that this drug be reserved for syphilis treatment in pregnant women and children.[4] Alternative treatment for syphilis in adult men and nonpregnant women includes a 2- to 4-week course of doxycycline (dependent on clinical stage of disease).[4] Our study revealed that in this high-risk group, men with a proven sexually transmitted cause of genital ulcer disease were more likely to present with primary syphilis rather than genital herpes compared with women, regardless of recent sex with 1 or more casual partners. This may in part be explained by sex differences in adherence to alternative therapeutic agents for syphilis, differential health-seeking behavior for genital ulceration, other sexual behavioral risks not examined or disclosed (such as sex with men), or differences in clinical presentation with genital ulcers being more apparent and visible on the external genitalia of males. In our study, 90% of primary syphilis cases detected by ulcer PCR were RPR seropositive, with the majority having titers consistent with a serological diagnosis of active syphilis. This suggests that incorporation of syphilis serology into GUS management algorithms, as an adjunctive diagnostic aid, would be a useful intervention.[16] The currently recommended criterion standard method for diagnosis of primary syphilis is a validated in-house or commercial *T. pallidum* nucleic acid amplification test of ulcer swab material, because of the lower sensitivity of syphilis serological assays, especially in very early disease.[2,17,18] In addition, clinical diagnosis of anogenital ulceration has been shown to be inaccurate in more than 50% of cases even by experienced clinicians.[17] The World Health Organization therefore recommends that, in a setting with limited molecular diagnostic or laboratory capacity, syndromic treatment is given for both HSV and syphilis to ensure same-day treatment.[18]

We have detected only sporadic cases of LGV through GUS surveillance over the last decade.[2] A vast majority of patients presenting to STI clinics at PHCs in South Africa self-report heterosexual orientation; we have therefore not observed the LGV resurgence attributed to *C. trachomatis* serovar L2b, manifesting as symptomatic proctitis among men who have sex with men in Western Europe.[19,20] Chancroid seems to have been eliminated as a cause of GUS: the last case detected through routine NICD surveillance at sentinel sites was in 2009.[2] Similarly, national STI etiological surveillance conducted in all provinces of the country failed to identify *H. ducreyi* in GUS specimens.[21] During a 12-year surveillance period, Giemsa-stained ulcer smears and an in-house PCR assay for *K. granulomatis* have not identified any cases of donovanosis, showing that it is not an established cause of GUS in our population.[5] Rare cases of donovanosis may be seen in clinical practice, and this etiology should be considered in patients presenting with refractory genital ulceration.[22] An etiological agent could not be identified in a significant proportion of GUS specimens. It is possible that some participants may have been exposed to incidental treatment for syphilis and that the ulcers were partially healed, although individuals with no identifiable ulcer pathogens were less likely to be syphilis seropositive. We did not specifically enquire about the recent use of antibiotics. Biopsy and histopathology may be useful in discerning non-STI or noninfectious causes, such as malignancy, trauma, vasculitis, and autoimmune disease.[23] This testing could be incorporated into a substudy as a component of future surveillance to better define ulcer etiology in South Africa. Limitations of our study include the small sample size of participants restricted to 3 sentinel sites, which limits generalizability of findings, and self-reported behavioral exposures that may be subject to reporting (social desirability) bias.

The prevalence of HIV in persons presenting with GUS is significantly higher than the prevalence in the general adult population of South Africa.[24] Interventions such as improved clinic- and community-based HIV testing services (HTS) with linkage to and retention in care are essential in ensuring improved screening uptake and viral suppression on antiretroviral therapy. Male medical circumcision has been shown to have a protective effect against both HIV and syphilis in heterosexual men and women.[25] In South Africa, successful community-based interventions to increase uptake of MMC have included home-based HTS followed by MMC reminders and referrals, as well as school-based MMC and HTS promotion and service access.[26]

In conclusion, HSV-2 remains the predominant cause of GUS in South Africa; however, there has been an increase in the relative prevalence of syphilis in recent years. It is essential to ensure adequate supplies of BPG at primary health care facilities for

syphilis treatment in persons presenting with GUS, as well as access to risk reduction services and integrated care such as MMC, HTS, condom promotion, syphilis serology, and partner notification and treatment.

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