CLINICAL REPORT

Herpes Simplex Virus Infection and Genital Ulcer Disease Among Patients with Sexually Transmitted Infections in Dar es Salaam, Tanzania

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The relative importance of *Haemophilus ducreyi* and *Treponema pallidum* in genital ulcer disease in Africa has decreased recently, whereas that of herpes simplex virus (HSV) type 2 has increased. We analysed 301 lesional specimens from Tanzanian patients with genital ulcer disease for the presence of *H. ducreyi*, *T. pallidum* and HSV-1/HSV-2 by performing a separate PCR for each pathogen. Infectious agents were detected in 211 (70%) of the cases. A single pathogen was found in 191 samples and two or more pathogens in the remaining 20. HSV-2 represented 83% of all identified pathogens, HSV-1 8%, *T. pallidum* 4% and *H. ducreyi* 5%. HSV-1 was identified as a single pathogen in four samples, in combination with others in an additional 14 samples. Thus, HSV-1 can also be the cause of genital ulcer disease in Africa. Regular surveillance of genital ulcer disease aetiology is important in programs for management of genital ulcer disease and HIV in Africa. *Key words: HSV-1; HSV-2; genital ulcer disease; STI; Tanzania*.

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Sexually transmitted infections (STI) often present as genital ulcer disease (GUD). STIs represent a worldwide problem, and the annual global incidence is estimated to exceed 20 million cases (1). Genital ulcers are more common in Africa than in Western Europe and North America (2). The leading cause of GUD in African countries used to be *Haemophilus ducreyi*, followed by *Treponema pallidum* (3, 4). However, the situation changed markedly from the late 1980s so that the relative importance of *H. ducreyi* and *T. pallidum* decreased significantly, whereas that of herpes simplex virus type 2 (HSV-2) increased (5, 6). Recent studies from Africa have confirmed that most genital ulcers are now caused by HSV-2 infection (7, 8). However, the aetiology of GUD may still show geographical variations and change from time to time within the same area.

HSV-2 is considered to be mainly sexually transmitted, and infectious virus can be shed during symptomatic as well as asymptomatic periods. In contrast, HSV-1 most often causes orolabial lesions, and transmission is primarily by non-genital personal contact. However, both viruses are capable of causing either genital or orolabial infection and can produce mucosal lesions that are clinically indistinguishable. Orolabial HSV-2 infections, however, are very rare. In recent years an increasing proportion of genital herpes in Western Europe and the USA is caused by HSV-1 infection (9–11), whereas HSV-1 does not seem to be the causative agent of GUD in Africa (5, 7).

The traditional strategy for managing GUD has relied on individual patient diagnosis and treatment. However, due to scarce laboratory facilities for diagnosing GUD in resource-poor settings, syndromic management has been the most commonly used approach in Africa. Selection of treatment is then based on which causative agent is expected to be most common in a particular area.

Different laboratory methods, such as direct antigen detection, virus isolation by cell culture and PCR, are commonly used for the diagnosis of genital herpes. PCR has markedly improved the laboratory diagnosis of genital herpes (12, 13) as well as of *T. pallidum* and *H. ducreyi* (4, 14, 15). Culture-based diagnosis of *H. ducreyi* or of HSV takes several days and may require facilities that are not always available when resources are limited. *T. pallidum* cannot be grown *in vitro* and detection of antibodies in the serum has relatively low sensitivity in primary syphilis.

Control of STIs has recently become a greater priority in global public health campaigns throughout the world, due to the association between STIs – particularly genital ulcers – and the acquisition of HIV infection (16–19). Although associations between HIV and chancroid or syphilis are statistically significant (20), the link with HSV-2 seems even more important (17, 20, 21). The latter relationship emphasizes the need for effective management of genital herpes as part of strategies for HIV control. Effective treatment of STI is indeed associated with a reduction in HIV infection (18).

In Tanzania, recent studies conducted on small population groups with genital ulcers suggest that HSV-2

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is the leading agent (7, 8, 22). The current study was designed to include larger numbers of Tanzanian patients at risk for STIs, to span a longer period of time, and to include HSV-1.

MATERIALS AND METHODS

Study population

Patients with GUD attending the Infectious Disease Clinic in Dar es Salaam, Tanzania between 1999 and 2001 were invited to participate in the study. Verbal consent was given by 319 consecutive patients. A total of 301 of these patients completed the study. All participants were interviewed for their responses to a verbal questionnaire. The study was approved by the Tanzanian ethics clearance committee.

Clinical specimens

Sterile Dacron swabs were used to collect material from the base of the ulcers. The swabs were immediately put into a liquid transport medium (Copan, Brescia, Italy) and stored at –80°C until further analysis. Serum samples were obtained for analysis for antibodies.

HIV antibody detection

HIV antibody detection was carried out by Behring Enzygnost Anti-HIV 1/2 Plus (Behring). Samples found to be positive were re-tested by Wellcozyme Recombinant HIV-1 ELISA (Abbot/ Murex, Wiesbaden-Delkenheim, Germany). All samples with discordant results in the two ELISAs were re-tested by Western blotting.

Preparation of positive controls for PCR

Laboratory strains HSV-1 17^+ and HSV-2 HG52 were grown in baby hamster kidney (BHK) cells, crude virus preparations obtained and the number of plaque-forming units per ml measured. DNA was extracted according to the method of Slomka et al. (12) and used as control in the PCR reactions.

H. ducreyi (strain D 160), kindly provided by the National Institute of Public Health in Oslo, Norway, was grown on chocolate agar. DNA was prepared from one colony of bacteria as described by Mohn et al. (23).

T. pallidum (Nichols strain) DNA was generously provided by Centres for Disease Control and Prevention, Atlanta, USA. The material was a crude extract from rabbit testicular tissue containing approximately 10^8 *T. pallidum* organisms per 50 µl.

PCR

DNA was extracted from the clinical samples using the QIA amp Mini Kit from Quiagen (Germany) according to the blood and body fluid spin protocol as described by the manufacturer. The sequences for all primers are specified in the quoted articles describing the PCR methods used.

Nested PCR for detection of HSV-1 DNA and HSV-2 DNA, respectively, were performed as described by Cinque et al. (24), except that the two reactions were run separately and the annealing temperature was reduced from 57°C to 55°C. The amplified products from the inner primers were 101 bp for HSV-1 and 139 bp for HSV-2.

Primers for detection of *H. ducreyi* were located to the 16S rRNA gene, and nested PCR was performed as described by Bruisten et al. (1), except that the primer concentration was reduced to 0.06 µM. The product from the inner primers was 309 bp long.

A 377 bp fragment of the DNA polymerase I gene (pol A) of *T. pallidum* was amplified by the method of Marfin et al. (15), except that betain was present (1 M) to facilitate the reaction and give more distinct bands in the gel.

The PCR methods detected 2–3 plaque-forming units (pfu) of HSV-1, 1 pfu of HSV-2, 4–5 pg of *H. ducreyi* DNA and approximately 20 DNA copies of *T. pallidum* DNA. Negative controls without DNA and positive controls containing the appropriate type of control DNA were included in each set of PCR reaction.

PCR products of correct sizes were identified by electrophoresis in agarose gels and comparison with a DNA ladder standard (Gene Ruler 100 bp ladder from MBI Fermentas, Lithuania).

Various combinations of PCRs were tested to detect two or more micro-organisms simultaneously. However, all combinations reduced the sensitivity compared with separate PCRs, which consequently were used for all four pathogens.

Statistical methods

To test the hypothesis of no bivariate association between identified pathogens and information given in the questionnaire, Pearson χ^2 test was used. When appropriate the strength of associations was estimated by calculating the odds ratio (OR). In analyses involving continuous variables (e.g. age, duration of ulcer) the χ^2 test for trend (linear by linear association) was applied. All statistical tests were performed at a significance level of 0.05.

Data analysis was performed using SPSS for Windows release 10.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Participants and data collection

The study included 319 persons, of whom 301 (91 women, 210 men, age range 17–60 years, mean age 29.9 years) responded to the questionnaire and allowed lesional sampling. The characteristics of the study population are shown in Table I. Nearly all of the participants identified themselves as heterosexual. The number of sexual partners during the last 12 months varied from 0 to 10, but 50% of the participants reported 2–4 partners. Females reported significantly lower numbers of sexual partners than did men. Approximately half of the participants denied any previous STI, and a similar number reported genital ulcers for the first time. The duration of the ulcers before attending the STI clinic varied from 1–2 days up to several weeks, but approximately 50% of the individuals had had ulcers for more than one week.

Detection of pathogens in lesional material

The detection of pathogens is based on the 301 patients responding to the questionnaire.

Table II shows that pathogens were detected in 211 (70%) of the patients. HSV-2 was by far the dominant agent. HSV-2 was present in lesional specimen in 64% of all participants (192/301) and in 92% of all cases

Table I. *Characteristics of the study population*

Response to questionnaire	Women	Men	Total
Answered (*n*)	91	210	301
Mean age (years)	28.7	30.4	29.9
Coitarchal age, years (mean)	16.4	17.6	17.2
Sexual preference (%)			
Heterosexual			97.3
Homosexual			0.3
Bisexual			2.3
Sexual partners last 12 months (%)			
0	9.0	3.4	5.1
1	58.4	26.6	36.0
2–4	32.6	61.8	52.9
5–10	0	8.2	5.6
Lifetime sexual partners (mean no.)			
0	2.2	0.5	1.0
1	48.4	10.5	22.0
2–4	41.8	51.7	48.7
5–10	6.6	30.8	23.4
> 10	8.8	32.8	25.1
Previous STI other than GUD (%)			
No	41.8	57.1	52.5
Yes	52.7	41.9	45.2
Do not know	5.5	1.0	2.3
Previous GUD (%)			
No	44.0	55.2	51.8
Once	17.6	26.2	23.6
Several times	38.5	18.6	24.6
Duration of the present ulcers (%)			
1–2 days	3.3	6.7	5.6
3–4 days	27.5	21.4	23.3
5–7 days	26.4	18.1	20.6
1–2 weeks	15.4	25.7	22.6
> 2 weeks	27.5	28.1	27.9
HIV seropositive (%)	55.7	40.8	45.3*

STI: sexually transmitted infection; GUD: genital ulcer disease. *p=0.019

with a single pathogen (175/191). In 4 cases HSV-1 was the only identifiable agent. Similar small numbers were observed for *T. pallidum* and *H. ducreyi*. In 20 cases (9.5%) there was a combination of two or more pathogens, one of these contained HSV-1, HSV-2 and *T. pallidum*. The most frequent combination was HSV-1 and HSV-2 (11 cases).

The relative importance of the 4 pathogens is shown more clearly in Table III, which lists all detections of a specified microbiological agent. Thus, the total number is larger than in Table II. HSV-2 constituted 83% (192/232) of all identified microbiological agents. Although the numbers for the other pathogens are small, HSV-1 was apparently at least as frequently identified as *H. ducreyi* and *T. pallidum*.

Table III. *Total number of infectious agents from genital ulcers*

	Detected	
Pathogen	*n*	%
HSV-1	18	7.8
HSV-2	192	82.7
Treponema pallidum	10	4.3
Haemophilus ducreyi	12	5.2
Total	232	100

HSV: herpes simplex virus

Analyses for associations between pathogen and demographic/behavioural factors

Very few statistically significant associations were found between identified aetiological agents or antibodies and demographic or behavioural factors (gender, age, coitarchal age, sexual preference, number of sexual partners (recent and life-time), marital status, educational level, duration and number of ulcer, previous STI or previous genital ulcer). Hence, the results are not tabulated, but will be discussed later.

DISCUSSION

This study analysed material from 301 genital ulcers in Tanzanian patients with STI, and infectious agents were identified by PCR methods in 211 cases (70%). Consistent with previous reports from Africa (5, 7) HSV-2 was found to be the dominant cause. This virus was detected in 91% of all cases in which one or more pathogens were identified (Table II), either as a single pathogen (175/191) or as a co-infection (17/20). HSV-2 was present in 64% of all patients with genital ulcers. The latter percentage is very similar to what has previously been reported from studies of smaller cohorts in the same area (7, 8). As in other African regions, *H. ducreyi* and *T. pallidum* were quantitatively of minor importance. One possible explanation for the relative decline of syphilis and chancroid is that the syndromic treatment approach has been targeted to bacterial, but not viral genital infections. Better access to a laboratory-based diagnosis of genital HSV infection in Tanzania might also have played a role.

The PCR methods used were slightly modified and also run separately, to increase their sensitivities and specificities. Nevertheless, no pathogen was detected in 30% of the ulcers. This is consistent with other reports

Table II. *Number of patients with single or multiple pathogens (n=211, 70%)*

Single pathogen (n = 191)				More than one pathogen (n = 20)						
HSV-1	HSV-2	Tp	Hd	HSV-1 + HSV-2	Tp+ HSV-1	Tp+ HSV-2	Tp+ Hd	Hd+ HSV-1	Hd+ HSV-2	HSV-1+ HSV-2+ Tp
4	175	3	9	11	1	4	1	1	1	1

Tp: *treponema pallidum*; Hd: *haemophilus ducreyi*; HSV: herpes simplex virus.

(8, 25). Ulcers with unidentified aetiology might be caused by pathogens other than those searched for in the present study. In 13% and 12% of the cases reported by Behets et al. (25) granuloma inguinale or lymphogranuloma venereum, respectively, was diagnosed clinically. Furthermore, the likelihood of defining an aetiological diagnosis might decrease with increasing duration of the ulcer, particularly in HSV infections. This was also evident in our patients where shorter duration of genital ulcer was associated with higher proportion of identified HSV-2 cases (p=0.002) (data not shown). Overall, in 1–4-day-old ulcers we could identify an aetiological agent in 79% of the cases, whereas this figure dropped to 66% if the ulcer had been present for more than one week (data not shown).

The trend in Western Europe and the USA, that HSV-1 has become increasingly important in genital infections, was now observed at a significant – but still low level (8%) – in Tanzania. This does not necessarily mean that the prevalence of genital HSV-1 infection in Tanzania is different from that in Europe. However, the detection of 4 (4/191) cases where HSV-1 was the single identifiable pathogen clearly shows that HSV-1 can be the causative pathogen in genital ulcers also in Africa, something that is very rarely reported from this continent (5, 7). We were not able to demonstrate any statistically significant age differences between HSV-1 and HSV-2 PCR positive patients. Males were less likely than females to have HSV-2 infection, however, the difference was not statistically significant (p=0.12) (data not shown).

We could not identify any demographic or behavioural differences between patients with one or more identified agents. Male patients seem more likely than female to have syphilis (OR 4.05) and chancroid (OR 5.00), however, the total numbers are small, and we suspect that this may be the reason why statistical significance was not reached (*T. pallidum*: 95% confidence interval (CI) 0.51–32.45, *H. ducreyi*: 95% CI 0.64–39.32). Neither could we demonstrate any other statistically significant demographic or behavioural factor (patient age, duration or number of ulcers, number of partners, previous STIs) characterizing patients with syphilis or chancroid. Again, small total numbers with syphilis and chancroid might be the reason. However, the absence of such associations could also question the validity of retrospective questionnaires.

Only 0.3% of the participants reported homosexual preference. Retrospectively, we realise that the questionnaire could have been more precise, as the term "homosexual" could be interpreted as exclusively homosexual activity, but also as ever having experienced sex with same gender. Homosexual and bisexual preference taken together was reported in 2.6% (Table I), which is in line with results from a previous study in the same cohort (26). Even 2.6% is a rather low figure, and there may be cultural, religious or other reasons that might explain a tendency to hide a homosexual preference in Tanzania. However, the study design does not allow any conclusive statements on this issue.

Almost half of the patients (45%, females (F) 56%, males (M) 41%) had antibodies against HIV-1 (Table I). By accident, the sera were lost before being analysed for antibodies against HSV-1, HSV-2 and *T. pallidum*. However, in a similar cohort of patients with STI in Dar es Salaam we have recently observed antibodies against HIV-1 in 33% (F 43%, M 26%) and against HSV-2 in 70% (F 77%, M 66%) (26, 27). Furthermore, in that cohort there was a strong and statistically significant association between HSV-2 seropositivity and HIV infection. The assumed higher prevalence of HSV-2 antibodies in women in the present study could – at least partly – explain the observed higher HIV seropositivity in the female participants. An association between HIV transmission and HSV-2 seropositivity has been shown in numerous studies (reviewed in (21)).

Epithelial disruption in symptomatic genital HSV-2 infection can be a portal of entry for HIV. However, in a prospective study in high-risk HIV-negative individuals, higher HIV transmission rates have also been demonstrated in HSV-2 seropositive individuals irrespective of genital lesions (28). Consequently, different or additional mechanism may be involved in the interplay between HSV-2 and HIV (29, 30). It has been shown that HSV regulatory proteins may upregulate HIV replication and thereby increase the titre of mucosal HIV shedding (29).

In the present situation with predominantly viral GUD aetiology, the approach of syndromic diagnosis and treatment, focusing on bacterial infections, will not be effective. The World Health Organization (WHO) has recently issued new guidelines for the syndromic management of genital ulcer disease, which include antiviral treatment for lesions consistent with genital herpes. Optimal management of HSV-2 infections, particularly in areas with a high prevalence of HSV-2 and HIV, is therefore important, including regular surveillance programs of genital ulcer disease. The present work indicates that such programs should also include HSV-1.

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