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Genital ulcers in Kenya

Clinical and laboratory study

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SUMMARY Of 97 patients with genital ulcers attending a special treatment clinic in Nairobi, Kenya, 60 harboured *Haemophilus ducreyi,* four herpes simplex virus, and five *Neisseria gonorrhoeae.* Eleven patients had serological evidence of syphilis; of these one case was confirmed by darkfield microscopy. In the remaining cases no aetiological agent was identified. An enriched chocolate agar with vancomycin and serum was a useful medium for primary isolation of *H ducreyi.* Tetracycline was generally ineffective in the treatment of ulcers, but sulfadimidine was successful in almost 80% of cases.

Introduction

Genital ulcers are a common problem in the tropics.[1] In the Special Treatment Clinic in Nairobi, Kenya, they constitute a major diagnostic and therapeutic challenge. Between 1976 and 1979 over 20 000 patients with genital ulcers were seen at this clinic. Of these, 18 245 were diagnosed clinically as having chancroid. Because of limited facilities routine laboratory investigations are not carried out. Dark-ground microscopy is not performed on ulcerative lesions, so the diagnosis of syphilis depends on clinical evaluation and serological results. Ulcers giving negative serological results are diagnosed as chancroid or as herpes genitalis on clinical appearance alone.

In May 1980, we began an investigation of randomly selected patients with genital ulcers. The purpose of this preliminary study was to determine the microbial aetiology of these lesions and their clinical and epidemiological features. Attempts were made to isolate *Haemophilus ducreyi* and evaluate the response of chancroid to two antimicrobial treatment regimens.

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Patients and methods

STUDY POPULATION

The first five patients with genital ulcers attending the clinic during one morning each week between May and July 1980 were selected for study. Age, sex, tribe, possible source of infection, and history of past sexually transmitted disease (STD) were noted. The number, size, features, and sites of ulcers and the presence of inguinal adenopathy were recorded.

LABORATORY INVESTIGATIONS

Material from the ulcer base was first cultured on enriched chocolate agar (ECA) and ECA with vancomycin 3 μg/ml (ECAV),[2] sheep blood agar, and MacConkeys medium in that order. Various sera (in a 5% concentration) including human, sheep, and fetal calf were added to the ECA and ECAV to facilitate the growth of *H ducreyi.* The plates were incubated at 36°C ± 1°C in a candle jar containing a moistened paper towel and read at 48 hours then daily for one week before the result was declared negative.

Presumptive identification of *H ducreyi* was made on typical colonial morphology—that is, colonies that moved intact across the agar surface and which, on Gram staining, showed Gram-negative rods occasionally with a typical "school of fish" appearance. Identification of these isolates was

confirmed by the University of Manitoba by published techniques.[3]

Three smears from the ulcer were prepared in random order for Gram staining for *H ducreyi* and Giemsa staining for *Calymatobacterium granulomatis.*

Scrapings from the ulcer base were obtained with a cotton swab, placed in virus transport medium, and transported on ice to the laboratory, where they were stored at −80°C until they could be inoculated into human amnion cells. Identification of herpes simplex virus (HSV) was based on cytopathic effect in tissue cultures.

Finally, specimens were taken for darkground microscopy, which was performed only on specimens from the last 26 patients, since the one available microscope was initially not functioning.

Serological tests for syphilis, including a Venereal Disease Research Laboratory (VDRL) test and a *Treponema pallidum* haemagglutination (TPHA) test, were performed on all patients at the initial visit.

In this preliminary study no attempt was made to isolate chlamydia from the ulcers and no serological tests for lymphogranuloma venereum (LGV) were carried out.

TREATMENT

The last 50 patients entered into the study were treated randomly (by means of a random number table) with either sulfadimidine 1 g four times daily or tetracycline 500 mg four times a day for seven days. If the ulcer had healed by day 7, no further treatment was given; if improved but not healed, treatment was given for a further week. If the ulcer failed to respond to treatment, the patient was given the alternative drug. If there was no response to either drug, streptomycin was added to the treatment regimen.

FOLLOW-UP

Patients were asked to return after seven and 21 days for re-examination. If the ulcer had not healed culture for *H ducreyi,* Gram staining, and serological tests for syphilis were repeated. If the lesion had resolved, only the serological tests were repeated.

Results

CLINICAL AND EPIDEMIOLOGICAL FEATURES

A total of 97 patients was entered into the study and all but two were male. Their average age was 25·6 years. Forty-four of the men were married, but only eight lived with their wives in Nairobi. Many of the men had migrated to Nairobi and were physically separated from their wives in the villages for long periods of time. The source of infection was usually reported to be prostitutes and only two men had had sexual contact with their wives. Most of the contacts occurred in Nairobi. Seventeen different tribes were represented but 76 of the 97 patients were either Kikuyu (25), Luo (30), Mkamba (8), or Luhya (13).

Fifteen of the men had a previous history of genital ulcers and 13 gave a past history of urethral discharge.

The incubation period of the genital lesion (calculated as the time interval from the most recent sexual contact to the appearance of the ulcer) varied from one day to more than one month. For 60% of the patients it was less than seven days. The time the ulcer had been present before treatment was sought was also extremely variable but on average was 10 days.

Fifty-five of the patients had a single ulcer, but the numbers ranged from one to more than six, with a mean of 2·2 ulcers. The most common sites for the ulcer were the penile shaft and coronal sulcus. Ulcers were also found, in order of decreasing frequency, on the prepuce, frenulum, tassel (skin remnant retained by some tribes including Kikuyu at circumcision), urethral meatus, and glans. In most patients with multiple ulcers the lesions were found at the same site, but six had ulcers at more than one anatomical site.

Inguinal adenopathy was present in 47% of patients; in 75% it was unilateral and in 25% bilateral. The presence of a bubo could not be correlated with either the size, number, or duration of the ulcers or whether or not the patient had been circumcised. Two men had fluctuant bubos, which were aspirated.

LABORATORY FEATURES

H ducreyi was isolated from the genital ulcer in 60 (62%) patients. In 22 of the 60 isolates identification was confirmed by appropriate tests at the University of Manitoba, Winnipeg. The remaining strains were not viable at the time of arrival in Winnipeg. The yield of positive culture results from enriched chocolate agar (ECA), ECA with vancomycin (ECAV), and ECAV incorporating different sera is given in table I. The addition of vancomycin to ECA improved the yield from 15% to 52%, of human serum to ECAV to 86%, of sheep serum to 98%, and of fetal calf serum to 100%.

Direct Gram staining of the ulcer material identified *H ducreyi* in 38 of the 60 patients who were culture-positive and in 18 of the 37 who were culture-negative. In patients in whom no characteristic Gram-negative rods were seen, over 50% of cultures grew *H ducreyi.*

Neisseria gonorrhoeae was isolated from the ulcer of three patients who had concomitant chancroid

TABLE I *Comparison of various media for primary isolation of H ducreyi*

Media	Culture results			
	Positive	*Negative*	*Total**	*% Yield*
ECA	3	17	20	15
ECAV	12	11	23	52
ECAV + 5% human serum	18	3	21	86
ECAV + 5% sheep serum	41	1	42	98
ECAV + 5% fetal calf serum	10	0	10	100

ECA = enriched chocolate agar; V = vancomycin
*Total number of patients with a final diagnosis of chancroid in whom this medium was tested

and from two patients in whom it was the only isolate from the ulcer. In all these patients the ulcer was located near the meatus, and three of them had a urethral discharge.

Herpes simplex virus was cultured from four patients; it was the only confirmed aetiological agent in three, whereas the fourth had reactive VDRL and TPHA test results. In a fifth patient, herpes was diagnosed clinically, but no HSV or any other organism was isolated.

Eleven patients had reactive VDRL and TPHA test results and were diagnosed as having syphilis on that basis. In six of these cases, including the one identified by darkfield microscopy, no other diagnosis was made. One patient harboured *H ducreyi* and another both HSV and *H ducreyi*. The remaining three patients were diagnosed clinically as having chancroid, but we were unable to isolate *H ducreyi*.

Calymatobacterium granulomatis was not identified in 97 Giemsa smears examined for Donovan bodies.

No definitive diagnosis was made in the remaining 23 patients with genital ulcers.

TREATMENT

Of the 51 patients who were randomly treated, 33 were confirmed by culture as having chancroid; of these, 16 were treated with sulfadimidine and 17 with tetracycline. Three of the former and three of the latter group failed to return for follow-up. The response to the two therapeutic regimens is shown in table II. Seventy-seven per cent of those treated with sulfadimidine had resolved or improved after seven days, whereas only 36% of those on tetracycline had improved. The number of patients remaining culture-positive at day 7 was also higher for the tetracycline group (50%) than the sulfadimidine group (23%).

Discussion

Many of the clinical observations in this study have been noted previously but the tribal distribution merits further comment. The Special Treatment Clinic in Nairobi is situated in a predominantly Kikuyu area, and a sample of 100 consecutive patients attending the clinic for any reason showed that the Kikuyu were the largest group (41%). However, they formed only 25% of the group with genital ulcers compared with the Luos, who comprise 20% of the clinic population but contribute 30% of genital ulcer disease. The Luos are the only tribe in Kenya which is traditionally uncircumcised, and chancroid is generally found to be more frequent in men who are not circumcised.[4 5] Clinical and epidemiological features of genital ulcers were not significantly different in the patients with chancroid than in those in whom no definite diagnosis could be established.

Most patients were young men who were either unmarried or physically separated from their wives, a situation similar to that which occurs during wartime.[6] The patients were mainly of low socio-economic class and acquired their disease from prostitutes. However, they were generally not promiscuous and admitted to only one or two recent sexual contacts. Unlike patients described in other studies, their personal hygiene was generally good.[6]

A definitive diagnosis of chancroid is notoriously difficult, since *H ducreyi* is a fastidious organism. In

TABLE II *Response to therapy in 33 patients with chancroid*

Treatment	No of patients					
	Treated	*Seen at 1 week*	*Healed*	*Improved*	*Not cured*	*Culture-positive at 1 week*
Sulfadimidine	16	13	1	9	3	3
Tetracycline	17	14		5	9	7
Total	33	27	1	14	12	10

the present study, no aetiological agent was identified in 23 patients who were clinically diagnosed as having chancroid; it is probable that many of these cases were chancroid. We did not find that a positive Gram-stain result correlated well with a positive culture result for *H ducreyi*. Since the Gram stain has often been used as a basis for the diagnosis of chancroid, we are continuing to evaluate its usefulness. The addition of sera to enriched chocolate agar improved growth for the primary isolation of *H ducreyi*. The highest isolation rate was obtained with 5% fetal calf serum (FCS). Others have found 10% FCS in heart infusion agar to be the best primary culture medium.[7] FCS is, however, expensive for routine isolation, and sheep serum may be a suitable alternative. With either serum, minimal growth is present at 24 hours but is very evident at 48 hours. In a few instances growth took as long as five days, and delayed appearance was associated with only a few colonies of *H ducreyi*.

Treatment with tetracycline was not very effective; similar results have been reported from Vietnam.[8] Sulfadimidine was more effective; this is not surprising since, on preliminary examination, many Kenya strains are resistant to tetracycline, whereas fewer are resistant to sulfonamides (unpublished data). With both treatment regimens, healing was slow and most patients required treatment for at least two weeks for complete healing. Trials of alternative drugs are indicated and are presently in progress.

This study has confirmed that at least the majority of genital ulcers suspected to be chancroid in Nairobi are due to *H ducreyi*. The availability of culture techniques to identify chancroid definitively will help in further studies to determine the epidemiology, the reservoir, and ultimately the control of this disease.

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