Isolation of *Haemophilus ducreyi* from genital ulcerations in White men in Johannesburg

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Summary

An analysis of sexually acquired genital ulcerations in 31 White patients who were examined microbiologically in Johannesburg showed *Haemophilus ducreyi* to be the causative agent in 23 (74%), whereas syphilis was the confirmed diagnosis in only 6 (19%). All the *H. ducreyi* isolates produced ß-lactamase. Previously, chancroid was considered to be an infrequent cause of genital ulceration in this population group. Unlike those with syphilitic ulceration, patients with chancroid usually do not respond to penicillin. The improved isolation procedures for *H. ducreyi* now enable the laboratory to confirm most cases.

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Chancroid, a sexually transmitted disease caused by *Haemophilus ducreyi*, has a world-wide distribution but is found more frequently in tropical and subtropical countries. Therefore, while Tan *et al.*[1] diagnosed 1 142 cases of chancroid during a 10-month period at a clinic in Singapore, only 55 cases were notified to health authorities in the UK during 1971. However, a rising incidence of the disease in Rotterdam has recently been reported,[2] and isolated outbreaks have occurred in Canada[3] and Greenland.[4]

Until recently, the diagnosis of chancroid was usually made on clinical grounds alone or by exclusion of other possible causes of genital ulceration such as syphilis, herpes genitalis and lymphogranuloma venereum (LGV). The only laboratory tests available, the examination of Gram-stained ulcer material, or isolation of the causative organism by the clot culture technique, were either nonspecific or relatively insensitive.

In 1978 Hammond *et al.*[3] described a culture technique which appeared to be superior to any other previously described. Using a well-defined selective medium they were able to isolate *H. ducreyi* from 8 of 16 suspected cases of the disease.

In this study we have used a modification of the medium described by Hammond *et al.*[3] to determine whether chancroid is a significant cause of genital ulceration in White men in Johannesburg.

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Patients and methods

The patients included in this study were referred for investigation of their genital ulceration by various practitioners in the Johannesburg area. During a 1-year period 31 White men with significant genital ulceration were examined. Of these, 5 (16%) had previously received antimicrobial therapy, usually with penicillin, with no clinical response.

Microbiological investigations

In each case material was collected from the genital lesion(s) and examined by dark-field microscopy for evidence of infection with *Treponema pallidum*. Calcium alginate swabs (Caligswab, Code 60-150-15; Inolex Corp.) were used to collect exudate from the bases of lesions for isolation of *H. ducreyi*. Specimens were plated directly onto the agar plates consisting of Müller-Hinton medium base (BBL) supplemented with 5% sterile horse blood, which had previously been heated to 75°C, 1% IsoVitaleX (BBL) and 3 μg/ml vancomycin (Eli Lilly). Inoculated plates were immediately placed in a CO_2 incubator which provided an atmosphere of 10% CO_2 in air and high humidity, and incubated at 37°C for 48 hours. In addition, blood specimens were collected for rapid plasma reagin (RPR) and fluorescent treponemal antibody absorption (FTA-Abs) tests for syphilis and the LGV complement fixation test (LGV-CFT). Tests for evidence of herpes virus infection were not performed.

Isolates were identified as *H. ducreyi* by the morphological and biochemical criteria described by Hammond *et al.*[3] and Kilian.[5] The chromogenic cephalosporin method was used to determine ß-lactamase production.[6]

Results

H. ducreyi was isolated from the genital lesions of 23 (74%) of the 31 patients. In these 23 patients there was no evidence of concomitant syphilis either on dark-field microscopy or by serological methods. The clinical appearance of the chancroid lesions of 1 such patient is shown in Fig. 1. The lesions of a further 6 patients (19%) showed infection with *T. pallidum* on dark-field microscopy. The diagnosis of syphilis was confirmed in each of these cases by positive serological tests (both RPR and FTA-Abs). As the LGV-CFT proved negative in all cases examined, no definitive diagnosis could be established in the 2 remaining patients (6%) who may still have suffered from chancroid despite failure to recover the organism. However, neither herpes genitalis nor granuloma inguinale can be excluded, as investigations for the agents responsible were not performed.

Discussion

The large number of cases of chancroid detected in this series was unexpected as the disease has previously been considered uncommon in Johannesburg. The high prevalence of chancroid

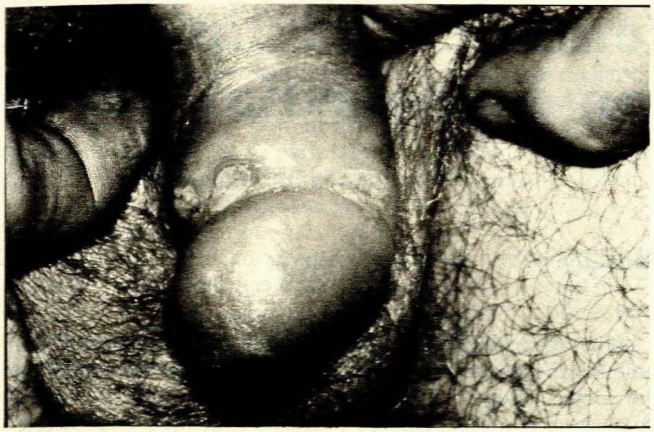

Fig. 1. Typical multiple chancroidal ulcerations.

(compared with syphilis) reported here may be more apparent than real and probably relates to the local practice of treating patients with genital ulceration with penicillin without referring them for microbiological investigation. As locally acquired cases of chancroid, unlike syphilitic chancres, are usually refractory to penicillin therapy, it is possible that the patient population of this study was biased in favour of those with ulceration due to resistance to penicillin. It is interesting to note that *H. ducreyi* was isolated from all 5 patients in this series who had received antimicrobial therapy before a laboratory diagnosis had been established.

Another reason for the high relative prevalence of chancroid in this study may be the greatly improved isolation procedure for *H. ducreyi*. The organism can now be isolated after 48 hours' incubation and be confirmed as *H. ducreyi* by its colonial appearance, cohesiveness, morphology on Gram staining and biochemical reactions. Herpes simplex virus is regarded as the commonest cause of genital ulceration in developed countries,[7] and although this probably also applies to White South Africans, reliable information on its incidence in this country is not available. It is, however, possible that, because of its characteristic clinical presentation, clinicians do not refer cases for virological examination and this may explain the absence of cases of herpes genitalis in this study. Laboratory confirmation of this condition may be readily obtained by culturing for the virus. In fact, it is now possible to make an accurate laboratory diagnosis in the majority of cases of sexually acquired genital ulceration with a dark-ground examination for treponemes, serological testing for syphilis and chlamydial infection and culture for *H. ducreyi*, *Chlamydia trachomatis* and herpes simplex virus.

A recent laboratory assessment of the susceptibility of 103 strains of *H. ducreyi* isolated in Johannesburg forms a rational basis for the treatment of chancroid in this city.[8] The vast majority (93%) were resistant to penicillin and ampicillin, and all produced a plasmid-mediated ß-lactamase, while most isolates were also insensitive to tetracycline and sulphonamides.[9] However, all the strains were fully sensitive to erythromycin and co-trimoxazole and these antimicrobial agents are probably the drugs of choice for the treatment of chancroid.

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