A microbiological study of genital ulcers in Kuala Lumpur

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Summary

The microbial aetiology of genital ulcers was studied in 249 patients (241 men and 8 women) attending a Sexually Transmitted Disease Clinic in Kuala Lumpur, Malaysia. Herpes simplex virus type 2 was isolated in 48 (19.2%) patients, *Haemophilus ducreyi* from 22 (8.8%), *Neisseria gonorrhoeae* from seven (2.8%) and *Chlamydia trachomatis* from four (1.6%). Syphilis was diagnosed in 18 (7.2%) patients on the basis of dark field microscopy. Two (0.8%) patients were found to have both chancroid and syphilis and one (0.5%) had both gonorrhoea and syphilis. No organism was isolated in the remaining 151 (61.5%) patients. Overall, the accuracy of clinical diagnosis was 58% for single infection, 67% for herpes, 63% for syphilis, 47% for chancroid and 0% for lymphogranuloma venereum. Therefore, our study confirms the need for laboratory tests to diagnose accurately the aetiology of genital ulcer disease.

Introduction

There are no readily available statistics on microbial aetiology of genital ulcers in Malaysia. The Sexually Transmitted Diseae (STD) clinic in Jalan Loke Yew, Kuala Lumpur, sees an average of 50 cases of genital ulcers per month.[1]

Genital herpes accounts for 40-80% cause of genital ulceration in developed countries but only 5-11% in developing countries.[2,3,4] In developing countries, 42-62% cause of genital ulceration is *Haemophilus ducreyi*, which accounts for only 1-2% in the United States.[2,5,6]

In Bangkok, Thailand, genital herpes was found to be the most common cause of genital ulcers in women and chancroid was the most common cause in men.[7,8,9]

The primary objective of this study was to determine the relative prevalence of the various micro organisms causing genital ulcers in patients attending the STD clinic, Jalan Loke Yew, Kuala Lumpur. An attempt was also made to corelate the macroscopic appearance of the ulcers with the various aetiological agents.

Patients and methods

Patients

Patients presenting with a first episode of genital ulcer to the STD clinics, Jalan Loke Yew, Kuala Lumpur, between June 1989 and June 1990 were included in the study.

Age, sex, ethnic group, possible source of infection and history of past STD were noted. Physical examination was performed and detailed description of the ulcer eg. number, size, feature and sites of ulcers and the presence of inguinal lymphadenopathy were recorded.

Laboratory Methods

Investigations performed on all patients included dark field microscopy (DFM) for *Treponema pallidum* and cultures for herpes simplex virus (HSV), *Haemophilus ducreyi* and *Chlamydia trachomatis*. Serological tests for syphilis and direct immunofluorescence (IF) test for HSV antigen were also performed.

The ulcer was first cleaned with sterile normal saline, and swabs were taken from the base of the ulcer for the following:

1. Culture of *H. ducreyi*
   The swab was inoculated directly on enriched GC Agar plates with 10% fetal bovine serum and 3 ug per ml vancomycin. The plate was immediately placed in a candle jar containing moistened cotton wool. In the laboratory the plate was incubated at 33°C in CO_2 with a piece of moist cotton wool. The plate was examined at 48 hours and thereafter daily for one week. *H. ducreyi* was identified by Gram stain and standard biochemical techniques.

2. Culture of HSV and direct IF test for HSV
   Swab taken from the base of the ulcer was immediately placed in viral transport medium consisting of Eagle's minimum essential medium and kept in ice until transferred to the laboratory. In the laboratory, the specimen was inoculated on to Vero cells and, was incubated at 37°C. Tissue culture was observed daily for one week for the characteristics cytopathic effects of HSV. HSV isolation was confirmed by immunofluorescence technique using flourescein isothiocyanate (FITC) labelled antisera against HSV 1 and 2 (Syva Microtrak Product, USA). Direct smears from ulcer was also carried out. Smear were fixed with acetone and direct IF test was carried out for HSV 1 and 2 (Syva Microtrak Product, USA).

3. Culture of *C. trachomatis*
   The transport medium used was the same as that for HSV isolation. The specimen was inoculated, with centrifugation, onto cycloheximide treated McCoy cells and was incubated in a 5% CO2 incubator at 37°C for 72 hours. After 72 hours, a second passage of specimen was carried out and reincubated for another two days before the cells were stained with IF stain against all the 15 known human serovars of *C. trachomatis* (Syva Microtrak Product, USA).

Darkfield Microscopy

After all the above swabs had been taken, the lesion was abraded to obtain exudate. A drop of exudate was placed on a slide and examined immediately under dark field illumination at a magnification of

x1000 by an experienced technologist and confirmed by the clinician. *T. pallidum* was identified by its characteristics morphology and motility[10] They were about 6-15um in lenght and 0.1-0.2um wide. They had rapid rotation about their longitudinal axis and flexing, bending, and snapping about their length.

Serological Tests
Rapid Plasma Reagin (RPR) test was performed according to standard techniques on all the patients sera using the Becton Dickinson Microbiology System, USA. The *T. pallidum* haemagglutination assay (TPHA) was performed with reagents from Fujirebio, Japan. Since the prevalence of untreated syphilis in this population is unknown, we elected to use darkfield microscopy for the laboratory diagnosis of acute syphilis.

Treatment
Patients suspected to have chancroid were started on co-trimoxazole two tablets twice daily for one week.

Those who were found to have positive DFM were started on intramuscular injection of benzathine penicillin 2.5 MIU weekly for two weeks.

Patients with a provisional diagnosis of lymphogranuloma venereum (LGV) were treated with tetracycline 500 mg six hourly for one week.

All patients were advised to clean their ulcers four times daily with normal saline. Treatment regimens were reviewed one week after the first visit, when all the laboratory investigation were ready.

Follow-up
Patients were asked to return after seven, 14 and 21 days for re-examination.

Data analysis
The index of suspicion for chancroid, syphilis, herpes and LGV were calculated as follows:

$$\text{Diagnostic Accuracy} = \frac{\text{No. of Correct Positive Clinical Diagnosis based on } \textit{laboratory confirmation}}{\text{Number of all Clinical Diagnosis for a particular agent}}$$

Results

Clinical findings
A total of 249 patients was seen, 241 were men and eight were women (Table I). More than 50% of them were between 21-30 years old. Sixty nine percent were single. Of these 249 patients 48% (119/249) were Indians, 37% Chinese and 13% Malays.

Of 168 patients with a past history of STD, 63% (105/168) had had previous episode of genital ulceration.

The source of infection in the men was usually reported to be prostitutes (85%). Three (38%) of the female patients seen, in this study, were prostitutes. All of the four (50%) married women denied sexual contact with men other than their spouses.

The mean incubation period of patients with *H. ducreyi* was 20.2 days, HSV 5.7 days,*C. trachomatis* 95.8 days and *T. pallidum* 34.9 days.

Table I
Demographic details of patients

Demographic Character	Men (241)	Women (8)	Total (249)
Age groups (yrs)			
16–20	19	0	19
21–25	63	2	65
26–30	62	1	63
31–35	42	1	43
36–40	20	3	23
41–45	13	0	13
46–50	11	1	12
> 50	11	0	11
Marital status			
Single	167	4	171
Married	74	4	78
Race			
Malay	28	4	32
Chinese	91	1	92
Indian	116	3	119
Others	6	0	6
Past history of STD	164	4	168
Past history of ulcer	103	2	105

About 46% (115/249) of the patients present with a sudden episode of genital ulcer and in 27% (67/249) the lesions started as vesicular lesions. Most (77%, 192/249) of the patients had painful ulcer. Thirty seven percent (92/249) patients had a single ulcer, while the rest had multiple ulcers ranging from two to 21 ulcers, with a mean of 3.4 ulcers. The most commons sites for the ulcer in the men were the penile shaft and coronal sulcus and in the women, the sites were the labia majora and labia minora.

The time the ulcer had been present before treatment was sought, was extremely variable but on the average was 20 days. One hundred and one (41%, 101/249) patients had received treatment before they were seen. In 24 (24%, 24/101) patients, these consisted only of topical application of antiseptic o antibiotic ointment. The other 75 (74%, 75/101) had both topical ointment application and oral medication.In some cases, injection was also given.

Laboratory Findings
The laboratory findings were as follows (Table II):

HSV type 2 was isolated in 48 (19.2%) patients.

This was followed by *H. ducreyi*,which was positive in 22 patients. Of these 22 patients, two of them also harboured *T. pallidum.*

Table II
Microbial aetiology of genital ulcers in 249 patients studied

	Organism isolated	No. of Men	No. of Women	Total
1.	Single infections:			
	HSV	45	3	48 (19.2%)
	H. ducreyi	20	0	20 (8.0%)
	T. pallidum	13	2	15 (6.0%)
	N. gonorrhoeae	6	0	6 (2.4%)
	C. trachomatis	3	1	4 (1.6%)
2.	Mixed infections:			
	T. pallidum & *H. ducreyi*	2	0	2 (0.8%)
	T. pallidum & *N. gonorrhoeae*	1	0	1 (0.5%)
3.	No pathogen isolated	151	2	153 (61.5%)
	TOTAL	**241**	**8**	**248 (100.0%)**

N. gonorrhoeaea was isolated from the ulcer of seven (2.8%) patients. One of these patients also harboured *T. pallidum.*

Eighteen (7.2%) patients had *T. pallidum* on DFM. DFM alone was positive in three, and in the rest it was positive together with RPR and TPHA tests. Three patients had mixed infection (mentioned above). Forty-one (16.5%) patients had reactive RPR and TPHA tests only.

Only four (1.6%) patients were found positive for *C. trachomatis.*

Correlation of clinical findings and aetiology

Table III summarises the clinical features and microbiological findings in the patients studied. Eighty five percent (41/48) of the patients with genital herpes had multiple and painful vesicular lesions, and only 15% (7/48) had single and painless ulcers. Only 33% (5/15) of the patients with syphilis had single and painless ulcers, and of these 5 patients, only 20% (2/15) had indurated ulcers.

Table IV compares the clinical diagnosis with the result of the microbiological investigations. A clinical diagnosis was made for 246 of these 249 patients. Three patients did not have an aetiology identified. In 96 patients it was possible to make a definite laboratory diagnosis. No aetiological agent was identified in 153 (62%) patients. Of these, 102 (66%, 102/153) patients were clinically diagnosed as having genital herpes infection, 38 (25%) patients had chancroid, and 2 (8%) patients had syphilitic ulcer.

Table V shows the index of suspicion and diagnostic accuracy for the 96 patients evaluated. The overall diagnostic accuracy for all pathogens tested was 58%.

Table III
Clinical features and microbiological, findings in 249 patients studied

Clinical Features	MICROBIOLOGICAL FINDING								
	HSV (n=48)	HD (n=20)	TP (n=15)	NG (n=6)	CT (n=4)	TP & HD (n=2)	TP & NG (n=1)	NIL (n=158)	TOTAL
Number of ulcer:									
Single	11	9	6	5	1	1	0	59	92
Multiple	37	11	9	1	3	1	1	94	157
Site*:									
Prepuce	14	3	3	0	1	0	0	27	48
Penile shaft	20	10	4	0	1	1	0	48	84
Coronal sulcus	9	10	8	3	1	1	1	39	72
Labia	2	0	2	0	1	0	0	1	6
Other site	26	11	6	4	1	0	1	48	97
Size:									
<1mm / pinpoint	16	4	8	0	1	0	0	25	54
1mm – 5mm	22	8	6	2	2	1	0	89	130
> 5mm	10	8	1	4	1	1	1	39	65
Edge:									
Shallow/erosion	45	15	10	5	4	2	1	141	223
Sloping	2	5	1	0	0	0	0	4	12
Punched out	0	0	2	0	0	0	0	5	7
Undermined	1	0	1	0	0	0	0	0	2
Everted	0	0	1	1	0	0	0	3	2
Tenderness	46	17	10	5	4	2	1	129	214
Induration	2	0	3	2	1	1	0	19	28
Secondary infection	7	6	2	2	0	1	0	31	49
Lympadenopathy	13	10	8	2	1	1	1	11	31

**multiple ulcers were sometimes located at different sites*
HSV = *herpes simplex virus*
HD = *H ducreyi*
TP = *T pallidum*
NG = *N. gonorrhoeae*
CT = *trachomatis*

Discussion

In this study HSV type 2 was found to be the most common cause of genital ulcers, and this was followed by *H. ducreyi*. HSV was present in 19% of patients, which is much lower than the 57% reported in Sheffield, England[2], 25.3% in Saint-Louis, Paris[11] and 40–80% in most developed countries;[2] and it is higher than the 12% reported in Bangkok,[12] 11% in Singapore,[13] 4% in Kenya,[3] 6% in Gambia[14] and 9% in Durban, South Africa.[6] Our finding is comparable to the 21% in Winnipeg[15] and 20% in Rwanda.[16]

H. ducreyi was isolated in 8.8% of our patients, this is comparable to the 13% reported in Sheffield, England[2] and 12% in Rwanda, but it is much lower compared to the 22% reported in Singapore,[13] 38% in Bangkok,[12] 52% in Gambia,[14] 40% in Durban, South Africa[6] and 62% in Kenya;[3] and slightly higher than the 1–2% in the United States[5] and 4% in Winnipeg.[15]

Table IV
Correlation between clinical diagnosis and microbiological findings in 249 patients studied

Clinical diagnosis	Nos.	Laboratory diagnosis*					
		HSV	HD	TP	CT	NG	NIL
Herpes	159	42	8	4	0	3	102
Chancroid	60	5	14	2	2	1	38
Syphilis	25	0	0	12	2	0	12
LGV	2	1	0	0	1	0	0
No diagnosis	3	0	0	0	2	0	1

* *Two laboratory diagnosis were made for three patients*

Table V
Comparison of clinical and laboratory diagnosis for the 96 patients with definite diagnosis

Diagnosis	Diagnostic Accuracy (%)
Herpes	67
Chancroid	47
Syphilis	63
LGV	0
Overall	58

Only 7.2% of our patients had *T. pallidium* infection. However, we are unable to compare this finding with the findings of the other studies because in our study only patients who are DFM positive were considered to have syphilitic ulcers. Two of these patients also had *H. ducreyi* isolated from their ulcers. This is not surprising, since these two common sexually transmitted diseases frequently coexist.[15,17,18] The low proportion of dark ground positive patients here should probably due to the high incidence of previous medication.

N. gonorrhoeae was isolated from 3% of patients; in three of these patients it was also isolated from the urethra. Its role as a pathogen in genital ulcers is uncertain. The possibility of contamination could not be ruled out in these patients.

LGV was not a common cause of genital ulceration, in our study. But, it must be remembered that ulcer is a transient phenomenon in chlamydial infections.

No aetiological agent was identified in 62% of our patients. The reason could be any of the following: (i) most of the patients had applied or taken medication for their ulcer; (ii) the low isolation rate may also be due to the relatively late presentation of genital ulcers in this clinic; (iii) out of these 102 patients who were clinically diagnosed as having herpes infection, 73 (72%) had similar past history of ulcer. In recurrent herpes infection, there are usually fewer lesions, less viral shedding and a shorter healing

time.[10] Also, it is known that the sensitivity of culture of HSV during the ulcerative phase of herpes is less than during the vesicular phase;[8,19] (iv) eight out of 12 patients whowere suspected of having syphilis had reactive RPR and TPHA tests but was negative for DFM. These eight patients could probably be having syphilis.

Our study also confirms the need for laboratory tests to diagnose accurately the aetiology of genital ulcer disease. For the 96 patients in whom an aetiological agent had been identified, the diagnostic accuracy was 58% only, which is lower than the 66% reported in Nairobi[18] and 68% in South AFrica.[16] The diagnostic accuracy varied from 67% for herpes, 63% for syphlis, 47% for chancroid and 0% for LGV. The diagnostic accuracy for herpes is higher than the 43% reported in Nairobi and 22% in South Africa. As for syphilis, our findings is also higher than the 42% reported in Nairobi and 55% in South africa. Our diagnostic accuracy for chancroid is lower than the 75% reported in Nairobi and 80% in South Africa.

Of the clinical characteristics examined, number of ulcers, site, size, edge, induration of ulcer base, presence of tenderness and inguinal lymphadenopathy were all not useful in predicting aetiology, except in herpes infection. However in herpes, once the vesicles have ruptured diagnosis becomes more difficult. The studies done in Winipeg and Amsterdam, showed that induration of ulcer base and absence of pain were found to be most useful in predicting the aetiology, but it was not so in our study. However, for herpes our findings were comparable to theirs.

From this study we can conclude that genital herpes is the most common cause of genital ulcer in the STD Clinic Jalan Loke Yew, Kuala Lumpur. This would mean that there is an urgent need for antiviral treatment and to identify effective strategies for control. Like other studies, we found that the clinical picture was an unreliable tool for the aetiological diagnosis of genital ulcers. Therefore, rapid laboratory tests would be desirable in the accurate diagnosis of genital ulcers, for appropriate management of such cases.

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