POLYMICROBIAL AETIOLOGY OF GENITAL ULCERS

V.S. Rajan
M. Doraisingham
E.H. Sng
A.L. Lim

INTRODUCTION

Genital ulceration is one of the commoner manifestations of sexually-transmitted diseases. The polymicrobial aetiology of these ulcers is well established (1) and it may be difficult to differentiate the different causes clinically. In the tropics, chancroid is generally regarded as being a common cause of genital ulcers (2), while in developed countries herpes progenitalis is more common.

In this study we have attempted to relook at the relative frequencies of the various causes of genital ulcers. The findings suggest that herpes progenitalis is not as uncommon as previously thought, and that the disease may manifest itself as a dirty ulcer. Also the serum culture technique (3) is too non-specific for the diagnosis of chancroid.

60 patients with genital ulcers were investigated to determine the aetiological flora of their ulcers. Six defaulted and were dropped from the study. All 54 were males between the ages of 16 to 56 and with an average of 24 years. 42 (77.8%) were single and 12 (22.2%) were married. 24 (44.4%) were unskilled workers, 17 (31.4%) were National Servicemen; 10 (18.5%) were skilled workers and 3 were from other vocations. All had exposed themselves to prostitutes within and outside the country.

MATERIALS AND METHODS

Following a full clinical examination, several specimens were collected from the ulcers and were inoculated directly on selective media for the cultivation of Neisseria gonorrhoeae and Haemophilus ducreyi. The Thayer-Martin medium was used for isolation of the gonococcus and the identification was made by colonial morphology, oxidase reaction and gram smears. Attempts to isolate the Haemophilus ducreyi was made on solid medium and pooled human serum (3). The solid medium used was a 15% chocolate agar enriched with Isovitalex and made selective in 3 ug/ml of vancomycin (4). Swabs from the ulcer were placed in freshly prepared Stuart's transport medium and sent to the laboratory within 2 hours for the culture of aerobes and anaerobes. Blood plate and Eosin Methylene Blue agar were used for aerobes while anaerobic blood agar was used for the anaerobes. One swab was also sent in Hank's transport medium together with a blood sample for herpes hominis virus isolation on human embryo lung fibroblast and embryonated hen's eggs, and serology by the complement fixation test. A second blood sample was sent at 2 weeks. Direct dark-field microscopy, and gram smears were taken on 3 successive days in the clinic. Blood samples for VDRL, FTA/ABS test were sent and repeated at 2 weeks, 6 weeks and 12 weeks.

FINDINGS

Clinical data of the cohort

17 (32%) had single ulcers and 36 (68%) multiple. The average number of multiple ulcers was 3.3. 21 (39.6%) of the ulcers were less than 5 mm; 20 (37.7%) between 5 to 10 mm and 12 (22.7%) more than 10 mm. 80% of the ulcers were dirty and 75% superficial. 11 (20.7%) were deep and 2 (3.7%) fungating. 39 (73.5%) were on the prepuce or subpreputial surface, 18 (34%) on the coronal sulcus; 10 (18.9%) on the frenum and 5 (9.4%) on the glans penis. Tenderness was severe in 7 (13.2%); moderate in 14 (26.4%), mild in 24 (45.2%) and no tenderness was elicited in 8 (15%). Significant inguinal adenopathy was present in 24.5%.

Middle Road Hospital
Singapore 0718
V.S. Rajan, MBBS, FRCP (E), FRCP (G),
DIP DERM (LOND)
Director and Senior Consultant Dermatologist
Clinical Associate Professor of Dermato-Venereology

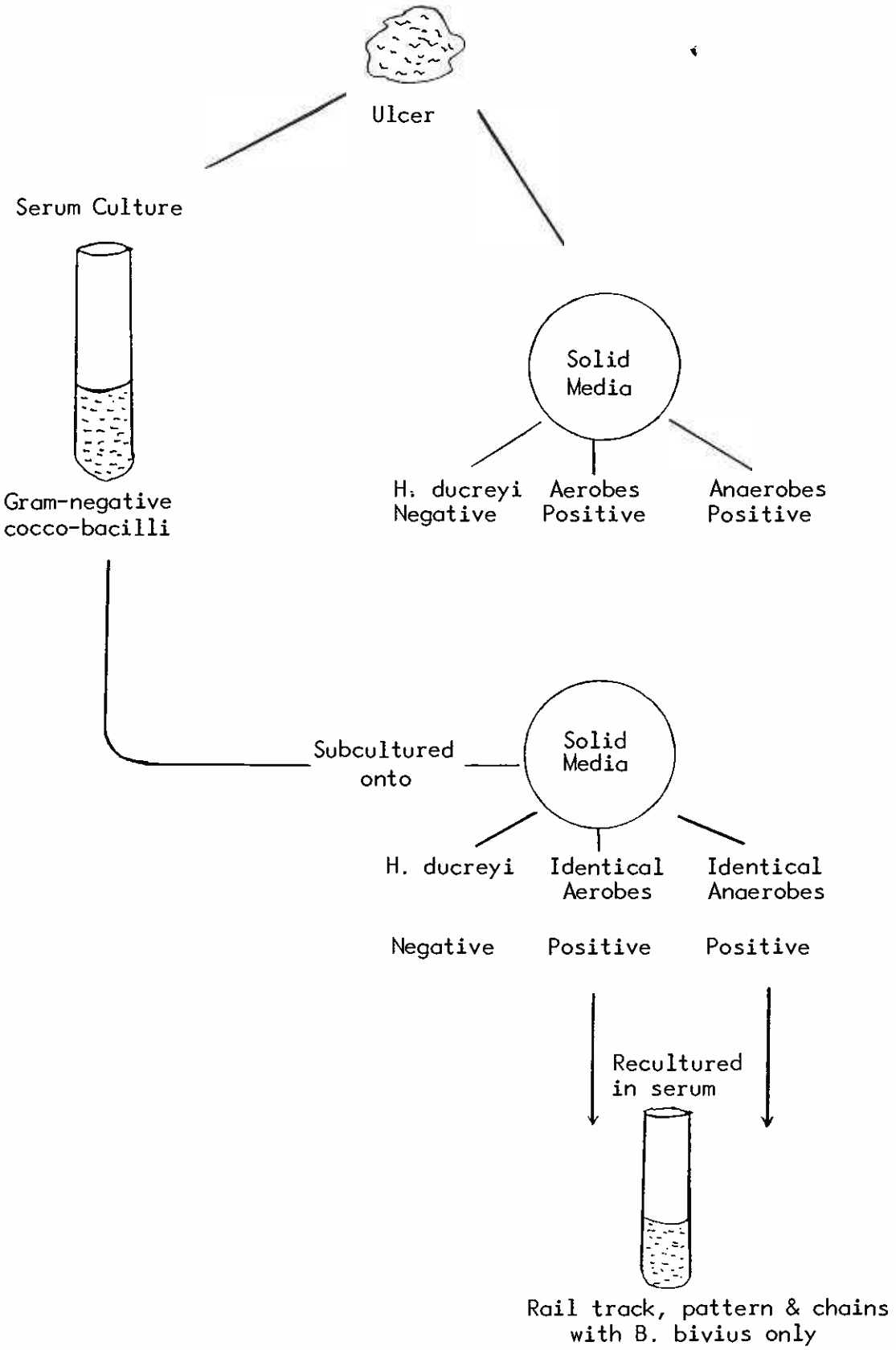
Department of Pathology
Singapore General Hospital
Singapore 0316

M. Doraisingham, MBBS, D. BACT.
Senior Virologist

E. H. Sng, MBBS, D. BACT
Senior Immunologist

A. L. Lim
Chief Laboratory Technician

Fig I


Ulcer
Serum Culture
Gram-negative
cocco-bacilli
Solid
Media
H. ducreyi
Negative
Aerobes
Positive
Anaerobes
Positive
Subcultured
onto
Solid
Media
H. ducreyi
Negative
Identical
Aerobes
Positive
Identical
Anaerobes
Positive
Recultured
in serum
Rail track, pattern & chains
with B. bivius only

Laboratory Findings

The most common laboratory finding was the gram negative bacilli in chains, both on gram smear and serum culture, which had a 100% correlation. The 'presumptive diagnosis' of H ducreyi infection was not supported by isolation of the organism on solid medium. Based on modern methods, the diagnosis of chancroid was not substantiated in this study. There were 21 positive cultures for aerobes, of which 7 were pure and 14 mixed with other organisms, including anaerobes, Herpes simplex, N gonorrhoeae and gram negative cocco bacilli in chains in serum culture. The aerobes isolated were Staphlococcus albus, Enterobacter aerogens, coliform organisms E coli, diptheroids and enterococcus. Anaerobes were isolated in 17 cases, of which 4 were pure, and the others were mixed with Treponema pallidum, Herpes simplex virus, Neisseria gonorrhoeae, aerobes and gram-negative cocco-bacilli in chains in serum culture. The most common organisms isolated were Bacteroides melaninogenicus and other bacteroides (Table I).

Aetiological Diagnosis

Based on well laid down conventional criteria, the diagnosis was possible in 22 of the 54 cases (40.7%) (Table II). Three had gonococcal ulcers, based on a positive culture for N gonorrhoeae, None of them had evidence of symptomatic urethritis. One of them had a positive culture for Herpes simplex type II and a rising H S antibody titre from 8 to 64 units. In all, Staphylococcus albus and anaerobes were isolated. Table III summarizes the findings of the 3 patients. Four patients had syphilis. The diagnosis was based on a positive serology with no past history of syphilis or a recent serological conversion. In none of the 4 patients was the dark-field microscopic examination positive. Two had associated Herpes simplex infection; in 2 anaerobes were isolated and in 2 Staphylococcus albus and diptheroids were isolated respectively. Table IV summarizes the findings of the 4 patients.

In 15 patients Herpes simplex infection was diagnosed based on isolation of the virus on culture and/or a rising H S antibody titre or a titre of 128 units, or more. In 9 herpes virus was isolated on culture and in 6 the diagnosis was based on serological grounds. Only in 2 of the 15, was there a past history of genital ulceration. In 3, there were no bateriological or serological evidence of other STDs. 2 had associated positive syphilis serology, 6 aerobes and 7 associated anaerobes. Table V summarizes the clinical and laboratory findinds of these 15 patients.

In 32 of the 54 patients (59.2%) no definite diagnosis was possible and the origin of their ulcers uncertain. However, in 26, gram-negative coccus-bacilli in chains, were isolated on serum culture. A more definite diagnosis of chancroid was not possible because of negative results on solid medium.

TABLE I

Laboratory Data of Cohort

	Test	No.	%
1.	Darkfield microscopy	0	0
2.	Positive VDRL serology or conversion	4	7.6
3.	Gram smears for intra, extra cellular gram-negative diplococci	0	0
4.	Culture — N gonorrhoeae	3	5.5
5.	Gram smears for gram-negative cocco-bacilli in chains	26	48.1
6.	"H. ducreyi" isolation on		
	(a) serum culture	26	48.1
	(b) solid culture	0	0
7.	Tzanck test for inclusion bodies/giant cells	0	0
8.	Herpes simplex isolation	9	16.6
9.	Rise in H.S. Antibodies or values at or above 128 titre	6	11.1
10.	Aerobic culture positive	17	31.4
	Aerobic culture positive pure	7	13
11.	Anaerobes culture positive	13	24
	Anaerobes culture positive pure	4	7.4
12.	Positive aerobes, anaerobes (pure)	4	7.4
13.	No organisms	2	3.7

TABLE II

Diagnosis of 54 Cases of Penile Ulcers

	Gonorrhoea	Syphilis	Herpes Progenitalis	Others including presumptive Chancroid
No.	3	4	15	32
%	7.4%	5.5%	27.8%	59.2%

TABLE III

Clinical and Laboratory Findings of 3 Patients with Gonococcal Ulcers

Patient	Incubation Period	Ulcers	Laboratory Findings
1	2 days	3 ulcers < 5 mm dirty, superficial painful, moderately tender	N. gonorrhoeae, bacteroides and Staphylococcus albus isolated
2	4 days	1 ulcer, 8 mm dirty, superficial moderately tender	N. gonorrhoeae, bacteroides and staphylococcus albus isolated
3	2 days	3 ulcers < 5 mm dirty, superficial severely tender	N. gonorrhoeae, bacteroides and Staphylococcus albus isolated H. simplex Type II isolated H..S antibodies 8-64 units

DISCUSSION

This study confirms the polymicrobial aetiology of genital ulcers. Like the previous study, the organisms isolated include definite aetiological agents like aerobes and anaerobes (1). We made no attempt to look for fungi, mycoplasma and chlamydia. Only 13 men had single organisms isolated from their genital ulcers. Two had H S virus, four anaerobes, seven aerobes and in two no organisms were isolated. In others a varying mixture of bacterial flora was observed.

A striking feature of this study was the singular absence of bacteriologically proven cases of chancroid. Previous studies from Singapore indicated that chancroid was the commonest cause of genital ulceration (2). The diagnosis in them were based on the demonstration of gram-negative cocco-bacilli in 'chains' on smear and serum culture. It has been reported that other bacteria such as streptococcus (5, 6) and B melaninogenicus (7) may give rise to similar appearances on serum culture. In many, the clinical features were consistent with chancroid. Thus direct microscopy or serum culture is not a reliable method of diagnosing chancroid.

A criticism of the serum culture technique developed by Borchardt and Hoke (3) is that its specificity was never tested. In this study specimens taken direct from the ulcers were inoculated into serum and also plated on selective solid media. Also when serum showed presumptive organisms, they were subcultured onto the solid medium. There was no correlation, we were unable to recover H ducreyi from the serum culture. However, aerobes and anaerobes were isolated. In addition to B melaninogenicus and other bacteroides, B bivius was isolated and identified by gas chromatography. On re-subculturing all the positive cultures back to serum, only B bivius produced the same pattern of gram-negative cocco-bacilli in chains or rail pattern (Fig. 1).

A high percentage of patients with dirty genital ulcers had herpes infections. Availability of culture facilities and complement fixation test for H S antibodies made this possible. In the absence of such facilities, a diagnosis of non specific genital ulcer or chancroid, would have been made. Contrary to experience in the developed world, herpetic ulcers here show a greater propensity to secondary infection to mask the correct diagnosis. In this series only 1 patient with herpes progenitalis of the 15 had a clean ulcer.

Four patients had syphilis. The diagnosis in two was made on late sero conversion at 2 and 4 weeks. In all 4 cases concomitant bacterial flora was shown, stressing the importance to repeat serological tests. However, the longer incubation period should alert the clinician of possible syphilis. Clinical features were atypical in all cases.

The finding of N gonorrhoeae in three patients without evidence of urethritis is of interest. The role of aerobes and anaerobes is unclear. Are they primary pathogens or secondary opportunistic invaders is yet to be clarified. In the laboratory we attempted to inoculate rabbits with the different aerobes and anaerobes isolated. A papule developed at the end of 48 hours with most strains but never progressed to frank ulceration (8). This is being followed up.

In conclusion genital ulcers have polymicrobial aetiology. Many dirty small genital ulcers mimicking dwarf chancroids may be herpetic ulcers with secondary infection. We

TABLE IV

Summary of Clinical and Laboratory Findings of 4 Patients with Syphilis

Patients	Incubation Period	Clinical Findings	Laboratory Findings
1	14 days	2 ulcers < 10 mm dirty, deep, mild tenderness. Soft No past history of ulcer	D G Negative VDRL negative to R4 FTA/ABS positive H.S. Type II isolated H.S. Antibodies 128-128 Bacteroides isolated
2	90 days	5 ulcers < 5 mm Mild tenderness Soft	D G Negative VDRL Neg-R2-R64 FTA/ABS positive Bacteroides isolated
3	60 days	10 ulcers < 5 mm Superficial, dirty Mild tenderness, soft	D G Negative VDRL R1 — R64 FTA/ABS positive Bacteroides isolated Staph albus isolated
4	16 days	2 ulcers < 5 mm dirty, deep mildly tender	D G Negative VDRL R64 FTA/ABS positive H.S. Antibodies 128-128 Bacteroides isolated Diptheroids isolated

TABLE V

Clinical and Laboratory Findings of Patients with Herpes Simplex Infections

Diagnosis		Incubation Period	Clinical Features	Other associated Lab. Findings
Culture	Serology			
1. Positive	Not done	4 days	1 painful mildly tender dirty ulcer < 5 mm	—
2. Positive	8-8	5 days	3 painful, mildly tender ulcers. 5-10 mm dirty & deep.	Staph albus diptheroids
3. Negative	8-128	3 days	4 painful, mildly tender ulcers 5-10 mm, dirty & deep.	Staph albus
4. Negative	128-128	14 days	Painless, mildly tender ulcer 10 mm with slough	Coliform VDRL R4 FTA positive
5. Positive	64-64	3	3 painful, mildly tender ulcers 10 mm, clean superficial	Bacteroides

TABLE V

Clinical and Laboratory Findings of Patients with Herpes Simplex Infections

Diagnosis		Incubation Period	Clinical Features	Other associated Lab. Findings
6. Positive	8-64	2	3 painful, severely tender ulcers 5 mm. Superficial, dirty.	Bacteroides Staph albus
7. Positive	32-128	6	2 painless but moderately tender ulcers 5 mm. Superficial & dirty.	Bacteroides
8. Positive	8-8	5	3 painful, mildly tender ulcers Superficial & dirty	Klebsiella
9. Negative	64-128	7 days	3 painful, severely tender ulcers 10 mm dirty & deep	—
10. Negative	64-128	35 days	3 painful, mildly tender ulcers. 5 mm. dirty & superficial. Past history of ulcers.	Bacteroides
11. Negative	128-128	6 days	2 painless, non tender ulcers. tender ulcers. 5 mm dirty & deep.	Bacteroides VDRL R64 FTA/ABS positive
12. Positive	128-128	21 days	Single painful, tender ulcer. 5 mm superficial & clean.	Staph albus
13. Negative	128-128	1 day	Single painless, mildly tender ulcer, 10mm Dirty & deep.	Bacteroides
14. Positive	8-64	3 days	2 painful, mildly tender ulcers 15 mm, dirty & deep.	Bacteroides
15. Positive	8-64	4 days	5 painless, mildly tender ulcers 5 mm dirty & superficial.	—

were unable to show that the demonstration of gram-negative cocco-bacilli on gram smears were presumptive diagnostic of H ducreyi infection. Though aerobes and anaerobes are commonly found, their aetiological role has still to be elucidated.

ACKNOWLEDGEMENT

We thank Y. A. Tan for her help in identifying Bacteroides bivius.

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