

The Etiology of the Non-Luetic Soft Chancre

Principal Investigators: M. Talmage M'cMinn, CPT, MSC
SP5 Alan T. Evangelista, B.Sc.

Associate investigator: Chiraphun Duangmani, M.D.

OBJECTIVE: To determine the bacterial etiology of penile lesions resembling non-luetic soft chancre.

BACKGROUND: Soft chancre or *ulcus molle* is the second most common venereal disease among US troops operating in Southeast Asia. The etiology of this disease is uncertain and treatment is occasionally misdirected because of this lack of bacteriological definition. The purpose of our study is to determine what bacteria are present in these lesions and to attempt to determine the causative agent.

DESCRIPTION: Specimens are obtained from penile lesions by means of scraping the floor of the lesion with a large gauge wire loop. Occasionally neglected ulcers must be washed to remove necrotic tissue before the edge and bottom of the lesion is scraped with the wire loop. A routine questionnaire is completed at the time of the examination, and venous blood is obtained for syphilis serology. 151 of 181 subjects included in this report were adult American males between the ages of 19 and 32 while the remainder were male members of the Thai military forces. Histories obtained at the time of examination indicated that none of the US personnel had treated themselves with antibiotics, while 65% of the Thai patients admitted prior treatment with non-prescribed drugs.

Lesions ranged from vesicular eruptions with no indication of bacterial infection through the classical 2-10 mm wide, slightly indurated, tender, deep ulcer described as the "soft chancre", to large fulminating lesions with marked destruction of tissue. Many patients presented with multiple lesions. We classified 34 lesions as "herpetic-like" which were characterized by painful multiple vesicular eruptions with a history of similar vesicular lesions that healed spontaneously. 68% of the patients with herpetic-like lesions reported prior lesions of a similar nature.

We had found from previous work that excellent growth of numerous organisms occurred on Eugonoagar plates with 25% fresh rabbit blood. This media was inoculated, as were Thayer-Martin media, sheep blood agar, desoxycholate agar, chopped meat broth, and fresh rabbit blood clot tubes, and were incubated at 37°C. Experience had revealed that inoculation of blood clot tubes made of the patients' blood did not add to recovery of any additional organisms. The Eugonoagar+25% rabbit blood plates, the sheep blood agar plates, and the Thayer-Martin media were incubated under increased CO₂ tension. Subsequent inoculation of another Eugonoagar plate with 25% fresh rabbit blood from the rabbit clot tube was made after overnight and 48 hour incubation. Three smears were made: one for immediate darkfield examination, another for gram stain, and the third for additional studies with special stains for cytology and presence of bacteria. No unusual cytology indicating viral involvement was ever observed in these smears.

RESULTS: During the period April 1971-April 1972, 181 penile lesions were examined and cultured in this laboratory. Table 1 presents the bacteriological findings from these cultures. Five of the 181 darkfield examinations revealed spirochetes and four serological examinations (RPR Card Test screen) were reactive. Only 7 specimens from patients were submitted for virology studies, determined by the nature of the lesion. Of these 7 patients with closed vesicles resembling herpetic lesions, 3 were found to harbour Herpes progenitalis.

Three of the "Haemophilus spp." we recovered from penile ulcers were sent to the diagnostic laboratories of the Center for Disease Control in Atlanta, Ga., in the United States. There, based on the inability of the organism to grow without haemolysis on rabbit blood and their need for factor V (phosphopyridine

nucleotide; DPN; NAD) and no need for factor X (haemin), the isolates were classified as Haemophilus parainfluenzae. These isolates were identical to the other Haemophilus spp. that we isolated from penile ulcers.

It is interesting to note that 3 isolates of Neisseria gonorrhoeae were isolated from penile lesions. All of these isolates of N. gonorrhoeae came from uncircumcised males and two of the lesions were on the shaft of the penis. One of these three males had gonococcal urethritis while the other two denied any symptoms of gonorrhoea. This finding may represent self-contamination with urethral exudate or may indicate infection of a pre-existing lesion with N. gonorrhoeae derived from an infected female.

Subcutaneous inoculations of pure cultures of the Haemophilus spp. into adult rabbits failed to produce any chancroid-like ulcers. Other pure cultures of bacteria isolated from penile lesions in our laboratory produced small self-limiting lesions but never anything resembling the penile ulcers from which they were isolated. Lesions produced by subcutaneous inoculation of the Haemophilus spp. were never positive on subsequent culture for the Haemophilus bacillus. Rabbits inoculated subcutaneously with mixtures of Haemophilus spp., diphtheroids, and nonhaemolytic streptococcus did produce ulcers resembling the typical soft chancre. We were able to recover all three of the bacteria inoculated on subsequent culture.

This laboratory has developed some additional biochemical test media that may enable additional characteristics of the fastidious Haemophilus organisms to be enumerated. By adding various amounts of NAD and/or haemin to sugar fermentation media and to other test media, we have begun to notice consistent growth patterns of the organisms recovered from the lesions in our study. This work continues and is somewhat dependent upon the quantity of Haemophilus isolates obtained.

DISCUSSION: While the etiology of soft chancre is usually defined in medical texts, a search of recent literature reveals that few workers have been successful in recovering the Ducrey bacillus. No adequate schema for its identification exists. To date, our data indicate that many penile lesions are infected with normal skin and/or vaginal flora. Many lesions are obviously herpetic eruptions that are characterized by multiple eruptions, pain, and recurrence. Patients presenting with this type of lesion usually reveal histories of past bacterial infections after neglect of the original lesion. Extreme care in this laboratory in obtaining thorough specimens and careful inoculation and incubation of a wide variety of media have not resulted in isolating a single organism that resembles the Haemophilus ducreyi bacillus that is generally defined as the causative agent for soft chancre.

Table 1.
Bacteriological Findings of Penile Lesions

	Sum	%
<u>Haemophilus spp.</u>	5	2.75
<u>Staphylococcus aureus</u>	83	45.65
<u>S. epidermidis</u>	84	46.20
<u>Micrococcus spp.</u>	3	1.65
diphtheroids	78	42.90
<u>Neisseria gonorrhoeae</u>	3	1.60
<u>Streptococcus faecalis</u>	20	11.00
beta streptococcus	6	3.3
alpha streptococcus	34	18.7
gamma streptococcus	81	44.55
<u>Escherichia coli</u>	14	7.7
<u>Klebsiella spp.</u>	2	1.1
<u>Enterobacter aerogenes</u>	5	2.75
<u>Enterobacter cloacae</u>	2	1.1
<u>Proteus spp.</u>	5	2.75
<u>Paracolonobactrum spp.</u>	4	2.20
<u>Pseudomonas aeruginosa</u>	3	1.65
<u>Alcaligenes spp.</u>	1	.55
<u>Herellea spp.</u>	1	.55
<u>Clostridium perfringens</u>	2	1.1
<u>Candida albicans</u>	1	.55