

CHLAMYDIA TRACHOMATIS INFECTION AND VENEREAL DISEASE

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Abstract. *Chlamydia trachomatis* was isolated by the irradiated McCoy cell technique from 44 out of 103 men with non-gonorrhoeic urethritis and from 11 out of 15 patients with post-gonococcal urethritis. In women attending the venereal diseases clinics, chlamydial infection was observed in 49 out of 130 patients (38%), an infection incidence of the same order of magnitude as the one noted for gonococcal infection (40%). In 19% both infections occurred simultaneously. Treatment with tetracycline eliminated symptoms and chlamydial infection in almost all cases. The significance of the findings is discussed.

Key words: *Chlamydia trachomatis*; Venereal disease

Several studies have been published describing the association of *Chlamydia trachomatis* infection with non-gonorrhoeic venereal disease (2, 7, 9, 13, 15). In men, chlamydial infection is rarely found without clinical signs of the urethral infection. In women the association of chlamydial infection with clinical symptoms is less obvious, although *C. trachomatis* has been isolated from 20 to 30% of women attending venereal diseases clinics (10). Thus chlamydia has been found in cases of acute salpingitis (6, 7), as well as in gonorrhoeic and non-gonorrhoeic cervicitis but also, in a low percentage, in clinically unsuspected women (10). The evaluation of the importance of *C. trachomatis* in genital infections has become a pertinent task. The present study describes the occurrence of chlamydia agents in first-time patients attending venereal diseases clinics in Göteborg.

MATERIAL AND METHODS

Patients. 245 men and women, who were attending for the first time the Department of Dermatology, Sahlgren's

Hospital, or the Outpatient Clinic for Venereal Diseases, Sociala Huset, Göteborg, were included in the survey. Specimens were taken in men from urethra and in women from cervix and examined for presence of *C. trachomatis*. Specimens collected with a blunt curette were added to tubes with 2 ml 2-SP medium (4). When possible, the specimens were inoculated the same day they were collected onto irradiated McCoy cells; otherwise they were stored frozen at -70° .

Laboratory procedures. A modification of the technique for isolation of chlamydiae originally described by Gordon & Quan (3) was used. So-called McCoy cells (a cell line shown to be of mouse origin) were cultivated and passaged in glass flasks in Eagle's MEM supplemented with 10% fetal calf serum, 2.5% glucose and 20 μ g/ml of gentamycin. The cells were irradiated at 4000 R from a ^{60}Co source and transferred to plastic cell culture plates containing 24 wells, each well with a flat bottom of 3 cm^2 .

The specimens were inoculated, each onto 4 wells, 0.25 ml per well. The irradiated cells were maintained in the same type of Eagle's MEM as described above. Before inoculation the cultures were rinsed twice with a solution of 30 μ g per ml of DEAE dextran in saline. Inoculated cultures were centrifuged at 2000 g for one hour. The cultures were then incubated at 35° for 2 hours, medium was changed and the cultures incubated for further 48 hours.

The first reading was performed at 48 hours after inoculation. The first of four wells, inoculated with one and the same specimen, was stained with Lugol's solution for 2 min. This stain was subsequently replaced with an iodine-ethanol-glycerol mixture (50 g KI, 50 g cryst. I_2 in 500 ml ethanol (95%) with 500 ml glycerol). The cultures were examined by ordinary light microscopy.

The second and third of the 4 inoculated wells were used for passage to a new set of cultures at 72 hours post inoculation. Cells and medium were collected, shaken vigorously with glass beads and 4 new wells with irradiated cells were inoculated, centrifuged and observed for another 72 hour period, when a third passage was performed. The inclusions of chlamydia were stained brownish by the iodine stain (Fig. 1).

Three of the isolated strains were kindly typed serologically by Dr John D. Treharne, London. They were all found to belong to *C. trachomatis*, serotype D.

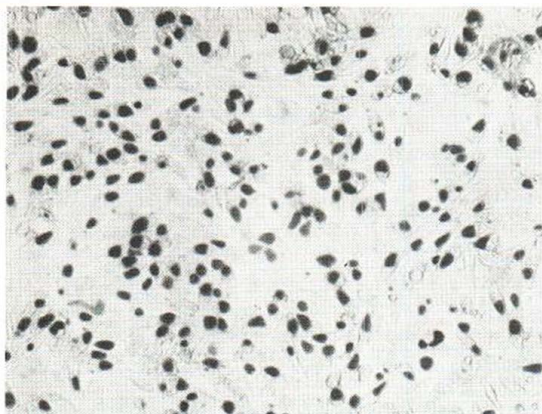


Fig. 1. McCoy cells inoculated 4 days previously with a specimen from a man with non-gonorrhoeic urethritis. The inclusion of *Chlamydia trachomatis*, rich in glycogen, are brownish stained with an iodine solution.

RESULTS

Men. 115 men with non-gonorrhoeic urethritis (NGU) were examined (Table I). All of them presented a history of urethritis with dysuria, urethral irritation and/or urethral discharge. Routine cultivation for demonstration of gonococci yielded negative results in all cases and microscopy of smears of urethral discharges failed to reveal the presence of *N. gonorrhoeae* in all cases.

Twelve specimens from these patients were discarded due to bacterial contaminations invalidating the cell culture test. In 44 of the remaining 103 male patients with NGU (43%), agents of *C. trachomatis* were isolated.

Treatment with tetracycline (0.1 g doxycycline daily for 7 days) of 22 patients with NGU resulted in 18 cases in disappearance of symptoms and the chlamydial infection. Three patients complained of irritated urethra even after the treatment, although the chlamydial infection had disappeared. A fourth patient, who received two consecutive treatments with tetracycline, became free of symptoms and chlamydial infection after the second treatment.

Among 45 men with gonorrhoea, treated with 1.4 g pivampicillin and 1.0 g probenecid, 15 developed post-gonococcal urethritis (PGU). Of this latter group, 11 patients (73%) were found to harbour a chlamydial infection. Thus, in total chlamydial infection was observed in at least 24% of the gonorrhoeic cases. In additional 20 men without clinical signs of urethritis, only one isolation of chlamydia was demonstrable.

Table I. Incidence of chlamydial infection in men with non-gonorrhoeic (NGU) or post-gonorrhoeic urethritis (PGU)

Disease	No. of		
	Patients	Isolations	%
NGU	103	44	43
PGU	15	11	73
Total	118	55	47

Women. Specimens were collected from 130 women attending the clinics due to fluor, genital irritation, or other symptoms of venereal infection, or called in as partners of men with venereal or suspected venereal disease. The patients were examined in the lithotomy position and specimens from urethra, cervix and rectum were examined for identification of *N. gonorrhoeae*. The cervical specimens were, in addition, studied for presence of chlamydia agents, trichomonas and candida (Table II).

Gonococcal infection was observed in 52 (40%) of the cases, chlamydial infection in 49 (38%). In 19% both types of infection were present simultaneously. In 24 women a chlamydial infection alone was demonstrable. Sixteen women out of 100 demonstrated trichomonas and of these 5 were found to have a chlamydial infection as well. From 9 out of 100 patients *Candida albicans* was cultivated and in 2 of the women concurrent infections with gonococci, chlamydia and candida were observed.

Patients with gonococcal infections were treated with 1.4 g pivampicillin and 1.0 g probenecid. The chlamydial infection cases received tetracycline (0.1 g doxycycline daily for 7 days). In 7 women with chlamydial infection alone, reexamined after one week, symptoms and infective chlamydia had both disappeared. However, 2 patients returned 3 to 4 weeks later complaining of fluor. Again *C. trachomatis* was observed as the sole infection. They both denied that there could have been any chance of reinfection. After a second period of treatment with tetracycline, symptoms and chlamydial infection disappeared.

In addition, 21 patients with gonococcal as well as chlamydial infections were controlled one week after treatment with antibiotics (penicillin and tetracycline), all proved freed of symptoms and infec-

Table II. Incidence of gonorrhoeic and chlamydial infections in 130 women attending clinics for venereal diseases in Göteborg

Infection	No. of patients	%
Gonorrhoea	52	40
Chlamydia	49	38
Gonorrhoea and chlamydia	25	19
Gonorrhoea and/or chlamydia	76	58

tions with the exception of one woman who seemed to harbour a clinically inapparent chlamydial infection.

DISCUSSION

The irradiated McCoy cell technique (3, 5) is considered to be several times more sensitive for the isolation of chlamydia than the previously employed yolk sac method (4). Improvements to the original cell technique have further increased its sensitivity (15). When applied to conjunctival material from patients suffering from TRIC infection of the eye, an isolation rate of 87% was reported (1). Of particular importance seems to be the storing at low temperature (-70°) of specimens and chlamydial strains and the use of centrifugal force to bring the chlamydia agents in close contact with the susceptible cells.

The irradiated McCoy cell technique has been used to evaluate both the clinical importance of chlamydial infection as a venereal disease and the epidemiology. In a survey of the literature presented by Oriel et al. in 1976, the percentage of chlamydial isolates from men with NGU varied from 23 to 57. In men with gonococcal infections the rate of isolation of chlamydia ranged from 11 to 32%, while in controls without urethritis, figures from nil to 5% were reported. The observations by the present authors of 43% of chlamydial infections in male patients with NGU, 24% chlamydial infections among cases of gonorrhoea, and findings of chlamydial infections in one only among 20 controls without urethritis agree well with previous findings.

The association of chlamydial infection with symptoms of disease in NGU urethritis strongly suggests an etiological relationship (10, 12). In contrast to the findings of Wallin & Gnärpe (17) we observed an association between symptoms of disease and genital chlamydial infection. Treatment

of NGU with tetracycline eliminated both the symptoms and the chlamydial infection and in patients with persistent symptoms of PGU treated with tetracycline the symptoms seemed to persist until the chlamydial infection disappeared.

In heterosexual as well as homosexual men with gonococcal urethritis the relative high rate of chlamydial infections has been considered as showing that the two infections are transmitted simultaneously (12). It also seems probable that a significant proportion of PGU is etiologically related to a genital chlamydial infection (11). Our findings would tend to support this assumption.

Men with chlamydial urethritis will, in most cases, probably receive treatment with tetracycline. On the other hand, women with genital chlamydial infection might well be discharged without treatment with tetracycline as no clearcut association of infection and symptomatology is at hand. In unselected female patients attending clinics for venereal diseases the rate of chlamydial isolations is often higher than in men. Hilton et al. (7) reported an incidence of 31% and Oriel et al. have reported isolations in 18%. The incidence of infection (38%) observed by us would indicate that chlamydial infection is just as common as gonorrhoea among Swedish women attending venereal disease clinics. The untreated infected female population might therefore be conceived of as the most probable source of chlamydial infection. Differences in rates of chlamydial isolations between promiscuous and non-promiscuous groups of women would support this assumption.

The incidence of combined gonococcal and chlamydial infection among female patients studied in the present report was 19%. Hilton et al. (7) found the simultaneous infection of gonorrhoea and chlamydia in not less than 63%. It has been suggested that chlamydia infection may survive better in the cervix than in the male urethra and that latent chlamydial infection may be reactivated by a gonococcal infection (15). With the small materials available it cannot, as yet, be stated whether this is true particularly with gonorrhoea or whether there exists a possible and similar reactivation of chlamydia following infections with trichomonas or candida agents.

Isolation of *C. trachomatis* from cervical secretions and the Fallopian tube has suggested a possible importance of chlamydial infection in the genesis of non-gonorrhoeic salpingitis (6, 7, 8).

However, it is possible that these findings merely reflect the relatively high incidence of mixed infections in female genital tract infections rather than an etiological relationship.

It should be noted that cytological abnormality of the cervix has been observed in association with chlamydial infection (10). As observed previously with herpes simplex virus type 2 antigen, serologic studies with chlamydial antigens have revealed a high prevalence of antibody in women with cervical dysplasia (16). In general, chlamydial antibodies were less prevalent in clinic populations other than women with dysplasia or cervical cancer (14). The background to the relative high incidence of some sexually transmitted infections in women with cytological abnormalities is poorly known. There are therefore several reasons for investigating the possible association of chlamydial infection with urethro-genital conditions.

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