

EPIDEMIOLOGY

Epidemiology of urogenital infections caused by *Chlamydia trachomatis* and outline of characteristic features of patients at risk

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Summary. A study of *Chlamydia trachomatis* infection was conducted in two stages on 15656 subjects at urogenital clinics of the Faculty of Medicine and Surgery at La Sapienza University in Rome, the S. Anna Hospital in Turin, and the Niguarda Hospital in Milan. The overall incidence of the disease was 6.4% in patients examined throughout the whole study period. The rate of positive cases was 5.8% for the 5270 patients examined up to 1990, and 6.7% for the 10386 patients examined from 1990 to 1992, showing an increasing trend. There was a much higher positivity rate in men (9.8%) than in women (6.0%); the difference was statistically significant. Of all patients, 60%, were asymptomatic. In symptomatic patients, *C. trachomatis* was present in 18.5% of cases of non-gonococcal urethritis and in 12.8% of cases of salpingitis. The highest incidence of *C. trachomatis* infection was in women who had begun sexual activity at an early age, (under 25 years in age), had several sexual partners and used intra-uterine contraceptive devices or spermicides or both.

Introduction

Four years ago, an initial study¹ in three large Italian metropolitan areas assessed the spread of *Chlamydia trachomatis* infections and studied their epidemiological characteristics. In a further study, the number of subjects tested was enlarged, the epidemiological characteristics of the infections were examined and an outline of characteristic features of the subjects at risk was obtained.

Materials and methods

Patients

Overall, 15656 patients, comprising 14025 women and 1631 men, were examined: 5270 patients were studied from 1988 until 1990¹ and 10386 were studied from 1990 to 1992. All attended urogenital clinics of the School of Medicine and Surgery of La Sapienza University in Rome, the Santa Anna Hospital in Turin and the Niguarda Hospital in Milan. The patients were divided into two groups on the basis of presence or absence of genitourinary symptoms—cervicitis, pelvic pain, "spotting", dyspareunia, dysuria,

salpingitis, leukorrhoea or leucoxanthorrhoea, in female patients; dysuria, proctitis, non-gonococcal urethritis (NGU), prostatitis, epididymitis or combined symptoms in male patients. Pregnant women and subjects needing surgical intervention were excluded from the study.

Each patient was assessed by completion of an epidemiological questionnaire, including the personal and medical history, and the following potential risk factors that could be correlated with disease attributable to *C. trachomatis*: present age, age at the time of first sexual intercourse, number of sexual partners during the previous 6 months and use of contraceptives.

Testing for *C. trachomatis* by an enzyme immunoassay

The Chlamydiazyme diagnostic kit (Abbott) was used for the direct detection of *C. trachomatis* antigen in endocervical swabs from women and urethral swabs from men.

Sample dilution buffer (1 ml) was added to the specimen and this was vortex mixed for 1 min in a test tube. Then 200 µl of a positive control, 200 µl of a negative control and 200 µl of each sample were pipetted into the wells in the reaction plate and one

Table I. Comparison between the frequency of cases of *C. trachomatis* infection in symptomatic and in asymptomatic subjects

Clinical category	Males		Females		All (males + females)	
	Number studied	Number (%) positive	Number studied	Number (%) positive	Number studied	Number (%) positive
Symptomatic	657	100 (15.2)	7613	520 (6.8)	8270	620 (7.5)
Asymptomatic	974	60 (6.2)	6412	319 (5.0)	7386	379 (5.1)
Total	1631	160 (9.8)	14025	839 (6.0)	15656	999 (6.4)

The statistical analysis was as follows. Difference in frequency of positive values; for males *versus* females: $\chi^2 = 35.8$; $p < 0.001$. Difference in frequency of symptomatic patients; for males *versus* females: not significant. Difference in frequency of symptomatic among positives; for males *versus* females: not significant. Difference in frequency of symptomatic among negative patients; for males *versus* females: not significant. Difference in frequency of positives among patients of the same sex; symptomatic *versus* asymptomatic males: $\chi^2 = 36.1$; $p < 0.001$; females: $\chi^2 = 21$; $p < 0.001$.

bead was added to each well. The reaction plates were incubated in a waterbath at 37°C for 1 h. If the sample contained chlamydiae, the micro-organisms would be absorbed to the bead.

After aspiration of any unbound material by washing with distilled water, 200 μ l of anti-chlamydial antibodies, which would bind to any chlamydial antigen on the bead in each well, were added and the reaction plates were again incubated at 37°C for 1 h. After washing, 200 μ l of antibody-enzyme conjugate containing horseradish peroxidase (HPR) was added and the beads were again incubated for 1 h at 37°C. The conjugate reacts with any antigen-antibody complex present on the surface of the bead.

After washing, the beads were transferred to test tubes and 300 μ l of *o*-phenylenediamine containing H₂O₂ were added to each tube. After 30 min at room temperature the reaction was stopped by adding 1 ml of 1 N H₂SO₄ to each well. The results were determined with a Quantum Spectrophotometer, to measure the absorption of each specimen at 492 nm.² Only cases positive by enzyme immunoassay (EIA) were tested by culture according to conventional methods³ and only those EIA-positive cases that were positive also by culture were regarded as chlamydia-positive.

Statistical analysis

The results were subjected to statistical analysis by the χ^2 test for comparison between frequency distributions, with Yates' correction for continuity.

Results

The overall incidence of the chlamydia infection was 6.4% in the 15656 patients examined throughout the whole study period; the rate of positive cases was 5.8% for the 5270 patients examined up to 1990, and 6.7% for the 10386 patients examined from 1990 to 1992.

A comparison between the sexes showed a much higher frequency among men (9.8%) than among women (6.0%), and these differences were statistically significant ($p < 0.001$) (table I).

Table II. Occurrence of positive cases of *C. trachomatis* infection in men and women with various clinical symptoms or with no symptoms

Symptoms	Number of patients	Number (%) of positive results	p value*
<i>Men</i>			
Inflammation	5	0	0
Dysuria	4	0	0
Proctitis	1	0	0
NGU	406	75 (18.5)	0.001
Prostatitis	145	15 (10.3)	NS
Epididymitis	41	5 (12.2)	NS
Combined symptoms	49	6 (12.2)	NS
None	743	63 (8.5)	0
<i>Women</i>			
Inflammation	3005	185 (6.1)	NS
Cervicitis	2653	179 (6.7)	0.01
Pelvic pain	824	89 (10.8)	0.001
"Spotting"	210	16 (7.6)	NS
Dyspareunia	456	37 (8.1)	0.05
Dysuria	359	44 (12.2)	0.001
Salpingitis	389	50 (12.8)	0.001
None	6412	319 (5.0)	0

NGU, non-gonococcal urethritis; NS, not significant.

* Statistical analysis of the differences compared to the frequency of positivity in asymptomatic men and women.

Presence of symptoms

The results (table I) indicate an overall higher frequency of positive findings in symptomatic patients (58.2%); the difference between sexes (40.2% for men, 54.2% for women) was not significant. Considering only the 999 positive cases of *C. trachomatis* infection, the incidence of symptomatic patients (620; 62%) was higher than that for all cases, or for that of the chlamydia-negative ones. According to symptoms and age, the frequency of positive *C. trachomatis* infections in male subjects was 15.2% for symptomatic patients and 6.2% for asymptomatic ones; for women, the percentages were 6.8% and 5.0%, respectively. The difference between the frequency of positive results in symptomatic and asymptomatic patients was statistically significant for both sexes ($p < 0.001$) (table I).

Table III. Frequency of positive results for *C. trachomatis* infection in relation to age in both symptomatic and asymptomatic subjects of either sex

Age years	Symptomatic patients			Asymptomatic patients			p value*
	Number positive	Number negative	Total	Number positive	Number negative	Total	
Men							
				<i>Absolute values</i>			
< 25	24	91	115	9	105	114	NS
26-35	47	275	322	34	500	534	0.001
> 36	29	191	220	17	309	326	0.01
Total	100	557	657	60	914	974	0.001
				<i>Relative values (%)</i>			
< 25	20.9	79.1	100.0	7.9	92.1	100.0	
26-35	14.6	85.4	100.0	6.4	93.6	100.0	
> 36	13.2	86.4	100.0	5.2	94.8	100.0	
Total	15.2	84.8	100.0	6.2	93.8	100.0	
Women							
				<i>Absolute values</i>			
< 25	166	1900	2066	89	1639	1728	0.005
26-35	233	2907	3140	151	2573	2724	0.05
> 36	121	2286	2407	79	1881	1960	0.001
Total	520	7093	7613	319	6093	6412	0.001
				<i>Relative values (%)</i>			
< 25	8.0	92.0	100.0	5.0	95.0	100.0	
26-35	7.4	92.6	100.0	5.5	94.5	100.0	
> 36	5.0	95.0	100.0	4.0	96.0	100.0	
Total	6.8	93.2	100.0	5.0	95.0	100.0	

NS, not significant.

* Statistical analysis of the differences compared to the frequency of positivity in asymptomatic men and women.

Sex

Table II indicates the difference in frequency of *C. trachomatis* positive reactions in relation to the symptoms and sex of patients.

The frequency of positive cases among men was higher (18.5%) in those with non-gonococcal urethritis and lower (10.3%) in those with prostatitis, whereas no positive results were detected in men with inflammation or dysuria or proctitis. The difference in frequency of positive cases, as between symptomatic and asymptomatic patients, was significant only for those with non-gonococcal urethritis.

Among symptomatic women, the frequency of positive results was higher in those with salpingitis (12.8%) and lower in those with pelvic inflammation (6.1%). The differences in positivity rates among asymptomatic female patients were all significant, except for those with "spotting" and inflammation (table II).

Age

Table III shows the incidence of proven *C. trachomatis* infection according to age group. The differences in positivity rates were similar for different age groups, although with different levels for men and women. These were: 20.9% of men and 8% of women aged up to 25 years; 14.6% and 7.4%, respectively, in those

aged 26-35; 13.2% and 5%, respectively, for those over 36 years old.

The incidence of positive cases among symptomatic patients was higher than for asymptomatic ones, for all age-groups. The differences were significant for all age-groups, except for men up to 25 years.

Age at first sexual intercourse

Table IV presents the distribution of female patients according to their age at first sexual intercourse, their positivity for *C. trachomatis* infection and their symptoms. The positivity rate for *C. trachomatis* infection was inversely related to their age at first sexual intercourse, being highest (12%) in women who had had their first intercourse at an early age (< 15 years). The differences according to the age of the first sexual intercourse, for both chlamydia positivity and presence of symptoms, were statistically significant.

Number of partners

The frequencies of chlamydia positivity (21.7%) and presence of symptoms (38.5%) were higher among women who had had sexual intercourse with more than one partner during the previous 6 months than for those who had had only one partner in that period (table V). These differences were statistically significant in both cases.

Table IV. Distribution of positive cases of *C. trachomatis* infection in women in relation to age at first sexual intercourse and to presence of relevant symptoms

Age at first sexual intercourse (years)	<i>C. trachomatis</i> test		Relevant symptoms*	
	Number examined	Number (%) of positive results	Number examined	Number (%) with symptoms
< 15	149	18 (12.1)	201	142 (70.6)
16-17	444	33 (7.4)	612	291 (47.5)
> 18	7986	543 (6.8)	6745	1485 (22.0)
Total	8579	594 (6.9)	7558	1918 (25.4)
	$p < 0.05^\dagger$		$p < 0.001^\ddagger$	

* See text.

† Difference in frequency of positivity among women in relation to age at first sexual intercourse.

‡ Difference in frequency of positivity and presence of symptoms among women in relation to age at first sexual intercourse.

Table V. Frequency of positivity for *C. trachomatis* infection in women and of relevant symptoms in relation to the number of sexual partners

Number of partners	<i>C. trachomatis</i> test		Relevant symptoms	
	Number examined	Number (%) of positive results	Number examined	Number (%) with symptoms
1	7140	371 (5.2)	5466	889 (16.3)
> 1	736	160 (21.7)	1368	527 (38.5)
	$p < 0.001^*$		$p < 0.001^\dagger$	

* Difference in frequency of positivity among women in relation to the number of sexual partners.

† Difference in frequency of positivity and presence of symptoms among women in relation to the number of sexual partners.

Table VI. Frequency of cases positive for *C. trachomatis* and of relevant symptoms in relation to different methods of contraception used

Contraceptive method	<i>C. trachomatis</i> test		p value*	Relevant symptoms		p value†
	Number examined	Number (%) of positive results		Number examined	Number (%) with symptoms	
Oral	2677	198 (7.3)	0.005	2295	818 (35.6)	0.001
Condom or diaphragm	1048	36 (3.4)	0.05	916	207 (22.6)	0.005
IUCD or spermicides	1163	95 (8.2)	0.005	973	256 (26.4)	NS
None	8623	470 (5.4)	...	6857	1930 (28.1)	...

NS, not significant.

* Difference in frequency of positivity among women using contraceptives or not; † difference in frequency to presence of symptoms among women using contraceptives or not.

Contraception

Table VI indicates the relationship between positivity for *C. trachomatis* and the use of different methods of contraception. The highest percentage of positivity occurred among those using an intra-uterine device (coil) or a spermicide (8.2%) and the lowest in those using condoms or diaphragms (3.4%). The differences in frequency of positive cases, between

women using and those not using contraceptives, were statistically significant.

Moreover, the frequency of symptomatic subjects varied in relation to the different methods of contraception used. The highest percentage was among those using oral contraceptives (35.6%) and the lowest in those using condoms or diaphragms (22.6%). The differences between the results for symptomatic women using contraceptives and the results for symp-

tomatic women who did not use them were statistically significant, except for those using intra-uterine devices or spermicides (table VI).

Discussion

As a result of the 4-year investigation, begun in 1988,¹ it is possible to draw some conclusions, regarding the epidemiological characteristics, spread and risk factors of *C. trachomatis* disease in Italy, and concerning the relationship of these findings to those reported in other countries.

There are no national data in Italy on the occurrence of chlamydial disease because, in most cases, such infections are neither revealed nor reported officially. The present study, carried out in two stages, has shown that the overall incidence of the disease was 6.4% over the whole study period—5.8% up to 1990, and 6.7% from 1990 to 1992.

From this research, it is clear that chlamydial infection has been increasing in the three areas of Italy studied. In many European countries, on the other hand, the number of positive *C. trachomatis* cases seems to be decreasing. In Sweden, where genital chlamydial infections have been recorded since April 1988, the occurrence of disease has decreased in recent years. In Austria, where prostitutes are inspected periodically for sexually-transmitted diseases, the occurrence of chlamydial infections has decreased from 20.4% in 1980 to 2.2% in 1989, notwithstanding a slight increase (3.3%) recorded in 1991.⁴ In the former Soviet Union, data from Moscow indicate a decrease in positive cases, albeit from an initially high incidence, from 34% in 1989 to 26% in 1990 and 20% in 1991.⁵ The trend of infection recorded in the rest of Europe is decreasing, whereas, as already noted, it is increasing in the three Italian areas. However, it is difficult to compare our findings with those in various other European countries, because data available on genital

infections caused by *C. trachomatis* varies according to sexual education, behaviour, and methods of prevention, diagnosis and treatment in different countries.

The trend that we have noted can be explained by taking into account the fact that epidemiological studies of the urogenital infections caused by *C. trachomatis* in Italy have become, in recent years, more specific, because of a better knowledge of the disease, improvements in diagnostic techniques, deeper understanding of major risk factors and better selection of patient samples. Moreover, our data show that a more relaxed attitude towards sexual activity, with associated lowering of age for first sexual intercourse and higher frequency of intercourse, both allowing a greater possibility of infection, contribute to the higher frequency of chlamydia-positive cases, both for men and women, in age groups up to 25. With regard to transmission, this has been facilitated by the fact that chlamydial urogenital infections are very often asymptomatic.

This research has shown that chlamydial disease in the three areas studied is asymptomatic in 60% of Chlamydiazyme-positive cases. This incidence is less than the 70% reported for the USA,⁶ but is more than the 5–20% reported in Sweden.⁴

The risk factors for infections caused by *C. trachomatis*, as reported elsewhere, are the same as for other sexually transmitted diseases, the main ones being sexual intercourse with many partners, or with only one who has promiscuous sexual habits.^{7–9} The present findings show that the risk is higher for women under 25 years of age (8.0%), who have had sexual intercourse before the age of 18 (12.1%), who have had many partners during the previous 6 months (21.7%), or who have used intra-uterine contraceptive devices or spermicides (8.2%). Thus, *C. trachomatis* infections seem to have affected the more sexually active women.

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