Indian J Med Res 136, December 2012, pp 1004-1010

Ocular *Chlamydia trachomatis* infections in patients attending a tertiary eye care hospital in north India: a twelve year study

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Received July 23, 2010

***Background & objectives*: Ocular infection with *Chlamydia trachomatis* is a major public health problem in densely populated countries like India. The true prevalence of such infections is uncertain due to insufficient data available from India. The aim of this study was to do a retrospective analysis of *C. trachomatis* eye infections in patients attending the outpatient department of Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, over a period of 12 years.**

***Methods*: From 1997 to 2008, the *Chlamydia* laboratory received conjunctival swabs from 1281 consecutive patients for *C. trachomatis* detection after thorough clinical examination. Specimens were subjected to direct fluorescent antigen detection assay using monoclonal antibody based commercial kit to detect the presence of C. *trachomatis* antigen.**

***Results*: Antigen positivity varied between 22-28 per cent. Children below 11 yr and people above the age of 60 yr showed comparatively higher antigen positivity (25.7 and 27.8%, respectively). As compared to males significantly (P<0.05) higher number of females in the age group of 31-60 yr were positive for *C. trachomatis* antigen. Patients with the clinical diagnosis of follicular/allergic conjunctivitis and trachoma showed higher rate of antigen positivity.**

***Interpretation & conclusions*: Northern India having dry and arid climatic conditions in most parts of the year was considered in the past as one of the trachoma hyper-endemic foci. The study indicated that laboratory proven *C. trachomatis* eye infection still persisted in this part of the country throughout the study period of 12 years.**

Key words Antigen detection - *Chlamydia trachomatis* - direct immunofluorescence assay - eye infections - trachoma

Ocular infection with *Chlamydia trachomatis,* especially trachoma, continues to be a major public health problem in many parts of the world. Recurrences often cause follicular/intense trachoma that may lead to trichiasis, corneal opacity and eventually blindness[1]. Despite the intense efforts by the World Health Organization (WHO) in the form of VISION 2020 and GET-2020, advocating multifaceted approach to interrupt transmission of *C. trachomatis*, the programme could truly be implemented only in 10 out of 55

trachoma endemic countries[2,3]. Reasons were attributed to lack of insufficient information from the densely populated countries like India and China[2-4]. Although the disease has been eliminated from many developed countries, a large population in Africa, Middle-East, South America, Asia, The Pacific, and Australia still suffer from this blinding disease that accounts for nearly 15 per cent of the global blindness[5].

India is a vast country with diverse socio-economic and varied hygienic conditions, and hence it is not feasible for a single laboratory or a single centre to undertake countrywide surveillance. In 1986-1989, a nationwide survey showed a decrease in prevalence of trachoma, prevalence rates of active trachoma during this period being 11.9, 7.84 and 6.56 per cent as opposed to 18.2, 44.1 and 45.1 per cent in 1962 in Punjab, Rajasthan and UP, respectively[6]. However, prevalence of blindness attributed to trachoma was 0.39 per cent in 1989[6]. Sporadic studies conducted in primary schools of Delhi in 1999 and 2004 reported that trachoma was the most common cause of ocular morbidity amongst the school going children[7,8]. Community studies were conducted in 1998 in reportedly hyperendemic areas of UP in 837 children and clinical trachoma prevalence was found to be 8.5 per cent[9]. In 2007-2008 community studies were carried out in hyperendemic areas of Haryana on 1000 children and active trachoma was detected in 4 per cent of them[10]. A part of the same study conducted in 1000 adult women, found evidence of cicatricial trachoma in 35 per cent of women above 30 yr of age[11]. Hospital based studies from south India reported *C. trachomatis* eye infection prevalence to be 17 per cent in 1990[13], 34.6 per cent in 1991-1992[13], 20.9 per cent in 1999[12] and 4.9 per cent in 2003[13]. Sporadic reports available on limited number of samples did not seem to be enough to measure the exact magnitude of the problem.

In special situations like high *vs.* low endemicity or active *vs* resolved trachoma cases, there are often wrong interpretations while correlating clinical assessment with laboratory findings, ultimately landing up either with incorrect laboratory diagnosis or incorrect clinical diagnosis. In an Egyptian study using ligase chain reaction (LCR) assay, 31 per cent of clinically active children did not have laboratory evidence of infection and 31 per cent infected children did not have clinical trachoma[14]. An Ethiopian study showed that the positive predictive value of clinical examination identifying infection was 66 per cent while inter-examiner variance was 30 per cent[15].

In the present study, we retrospectively analysed the information on all patients tested for the presence of *C. trachomatis* infection for the past 12 years (1997-2008). All consecutive patients likely to have *C. trachomatis* eye infections coming to a tertiary care eye hospital in north India during this period were tested using direct immunofluorescence assay (DFA), which is being routinely carried out in the Chlamydia laboratory.

Material & Methods

A total of 1281 patients (763 females, 518 males) in the age-group of 1-90 yr were referred to the *Chlamydia* laboratory from the outpatient department (OPD) of Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi, for the laboratory confirmation of *C. trachomatis* eye infections from 1997 to 2008. Study protocol was approved by the AIIMS ethics committee. All consecutive patients coming to hospital during this period with acute or chronic follicular conjunctivitis and keratoconjunctivitis were included. Patients with frank purulent conjunctivitis and acute haemmorrhagic conjunctivitis coming during viral conjunctivitis outbreaks were not included.

Prior to referring the patients to our laboratory, experienced ophthalmologists performed clinical examination using slit lamp biomicroscopy of the anterior segment of the eye and ocular adenexa. WHO simplified diagnosis and grading system for trachoma was used in patients for diagnosis of clinical trachoma wherever it was possible[4].

Specimens from the superior/inferior palpebral conjunctiva of both eyes were taken with sterile wet cotton swabs and smeared onto a clean Teflon-coated glass slide (one specimen from each eye). The slides were air-dried, fixed in cold acetone for 10 min and subjected to direct immunofluorescence assay for *Chlamydia* antigen detection.

Direct immuno-fluorescence assay (DFA): The monoclonal antibody based *C. trachomatis* direct specimen kit (MicroTrak, USA) was used for the detection of antigen using the standard protocol[16]. Briefly, conjunctival smears were covered with 30 µl of fluoresceine-isothiocynate (FITC)-conjugated murine monoclonal antibodies to *C. trachomatis* for 30 min at 37^{o}C in a humidified chamber. The slides were washed with double distilled water, air-dried, mounted and observed under the fluorescent microscope (Nikon, Japan). The positive-control was fixed mammalian cells

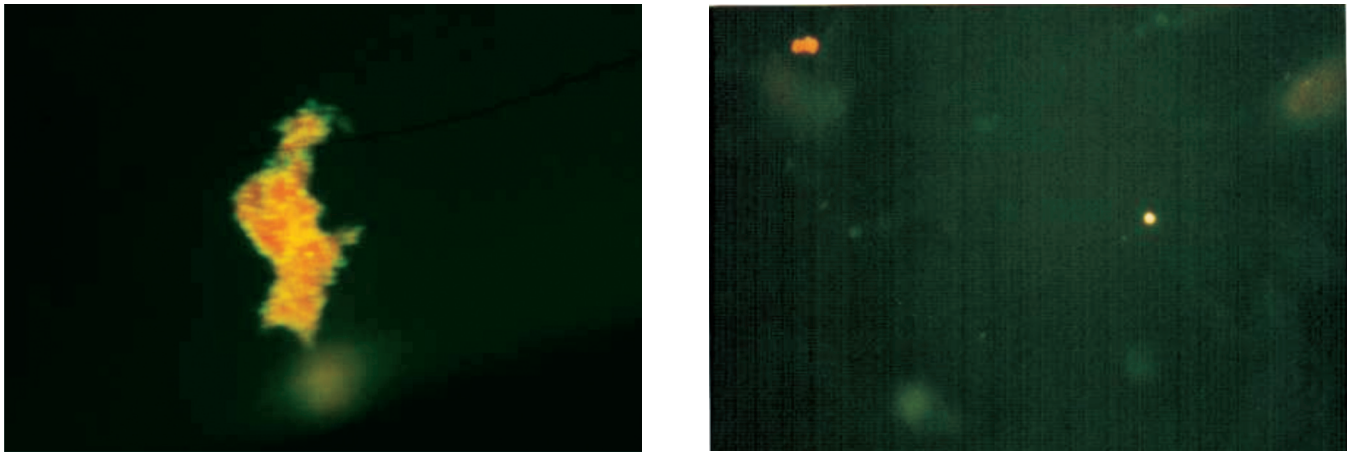

Fig. 1. *C. trachomatis* particles observed under direct immunofluorescence assay (1a: x400, 1b: x1000).

containing *Chlamydia* elementary/reticulate bodies, (EB/RB) (provided with the kit) while the negative control contained normal uninfected mammalian cells. *Chlamydia* elementary bodies appeared as round, bright, apple green, fluorescent particles, regular in outline (Fig. 1).

Statistical analysis: Antigen positivity was summarized by frequency (percentage). Test of proportions (Z-test) was used to compare differences in percentage antigen positivity amongst males and females for each age group separately. Also test of proportions (Z-test) with Bonferroni correction was used to compare *C. trachomatis* antigen positivity amongst the groups with various clinical diagnosis. STATA 11.0 statistical software was used for data analysis.

Results

Of the total 1281 patients (763 males, 518 females age 1-90 yr) studied, 485 were clinically diagnosed as trachoma, 296 as cases of follicular, acute or allergic conjunctivitis, 94 kerato-conjunctivitis and the remaining 406 were labelled as probable cases of other eye infections/manifestations. Of the 1281 patients studied, 321 (25.05%) were found positive for *Chlamydia* antigen. Children below 11 yr of age and older people above the age of 50 yr showed comparatively higher positivity for chlamydia antigen (25.7, 25.0 and 27.9%, respectively) (Table I). Significantly larger number of females 222 (29.1%) were positive for *C. trachomatis* antigen than males 99 (19.1%). Females in the age group of 31-60 yr showed significantly higher rate of antigen positivity ($P<0.05$) as compared to males.

Although, the range of *Chlamydia* antigen positivity remained between 22-28 per cent during the study period, overall antigen positivity was above 21 per cent in our study population over a 12 year period, with higher rates in the years 1998, 2002 and 2004 (Table II and Fig. 2).

Table I. Age and sex distribution of *Chlamydia* antigen positivity

Age group (yr)	Males		Females		Total	
	No. of specimens	Ag Positive (%)	No. of specimens	Ag Positive (%)	No. of specimens	Ag Positive (%)
1-10	71	14 (19.7)	104	31 (29.8)	175	45 (25.7)
11-20	97	19 (19.6)	144	32 (22.2)	241	51 (21.1)
21-30	65	11 (17.0)	101	27 (26.7)	166	38 (22.9)
31-40	71	14 (19.7)	99	34 (34.3)*	170	48 (28.3)
41-50	69	12 (17.4)	102	32 (31.4)*	171	44 (25.7)
51-60	67	11 (16.4)	101	31 (30.7)*	168	42 (25.0)
>60	78	18 (23.0)	112	35 (31.2)	190	53 (27.9)
Total (1-90)	518	99 (19.1)	763	222 (29.1)**	1281	321 (25.05)

P *<0.05, **<0.001 compared to males

Table II. Year-wise break up with age groups for patients with *Chlamydia* antigen positivity

Age (yr)		Years												
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
	Tested	17	12	18	7	9	9	8	12	17	11	22	33	175
1-10	Positive (%)	4(23.5)	3(25.0)	3(16.6)	2(28.5)	2(22.2)	5(55.5)	5(62.5)	3(25.0)	5(29.4)	3(27.2)	7(31.8)	3(9.0)	45(25.7)
	95% CI	6.8, 49.9	5.5,57.2	3.6,41.4	3.7,71.0	2.8,60.0	21.2,86.3	5.6,57.2	24.5,91.5	10.3,56.0	6.0,61.0	13.9,54.9	1.9,24.3	19.4, 32.9
	Tested	31	9	11	14	19	12	14	19	22	24	33	33	241
11-20	Positive (%)	7(22.5)	2(22.2)	2(18.1)	3(21.4)	5(26.3)	1(8.3)	3(21.4)	3(15.7)	3(13.6)	8(33.3)	6(18.1)	8(24.2)	51(21.1)
	95% CI	9.6, 41.1	2.8,60.0	2.3,15.8	4.7,50.8	9.1,51.2	0.2,38.5	4.7,50.8	3.4,39.6	2.9,34.9	15.6,55.3	7.0,35.5	11.1,42.3	16.2, 26.9
	Tested	15	7	8	13	7	16	11	12	3	17	38	19	166
21-30	Positive (%)	2(13.3)	1(14.2)	3(37.5)	2(15.3)	1(14.2)	3(18.8)	2(18.1)	3(25.0)	1(33.3)	3(17.6)	9(23.6)	8(42.1)	38(22.9)
	95% CI	1.7, 40.5	0.4,57.9	8.5,75.5	1.9,45.4	0.4,57.9	4.0,45.6	2.3,51.8	5.5,57.2	0.8,90.6	3.8,43.4	11.4,40.2	20.3,66.5	16.7, 30.0
	Tested	10	3	8	11	15	14	10	11	8	17	35	28	170
31-40	Positive (%)	3(30.0)	2(66.6)	2(35.0)	2(18.1)	2(13.3)	4(28.6)	4(40.0)	4(36.3)	2(25.0)	5(29.4)	10(28.6)	8(28.6)	48(28.2)
	95% CI	6.7, 65.2	9.4,99.2	3.2,65.1	2.3,51.8	1.7,40.5	8.4,58.1	12.2,73.8	10.9,69.2	3.2,65.1	10.3,56.0	14.6,46.3	13.2,48.7	21.6, 35.6
	Tested	11	4	8	12	16	15	9	10	9	16	31	30	171
41-50	Positive (%)	1(9.0)	1(25)	1(12.5)	2(16.6)	2(12.5)	4(26.6)	3(33.3)	5(50.0)	2(22.2)	6(37.5)	8(25.8)	9(30.0)	44(25.7)
	95% CI	0.2, 41.3	0.6,80.6	0.3,52.7	2.1,48.4	1.6,38.3	7.8,55.1	7.5,70.1	18.7,81.3	2.8,60.0	15.2,64.6	11.9,49.4	14.7,49.4	19.4, 33.0
	Tested	11	4	7	11	12	12	10	12	10	14	30	35	168
51-60	Positive (%)	3(27.2)	1(25.0)	3(42.8)	3(27.2)	2(16.6)	3(25.0)	2(20.0)	3(25.0)	2(20.0)	3(21.4)	9(30)	8(22.8)	42(25.0)
	95% CI	6.0, 61.0	0.6,80.6	9.9,81.6	6.0,61.0	2.1,48.4	5.5,57.2	2.5,55.6	5.5,57.2	2.5,55.6	4.7,49.4	14.7,49.4	10.4,40.1	18.7, 32.3
	Tested	11	1	5	18	11	25	14	9	1	16	47	32	190
>60	Positive (%)	4(36.6)	1(100)	0	8(44.4)	6(54.5)	9(36.0)	1(7.14)	2(22.2)	1(100)	2(12.5)	7(14.8)	12(37.5)	53(27.8)
	95% CI	10.9, 69.2	2.5,1.0	0.0,52.2	21.5,69.2	23.4,83.3	18.0,57.5	0.2,33.9	2.8,60.0	2.5,1.0	1.6,38.3	6.2,28.3	21.1,56.3	21.6, 34.8
	Tested	24/106	11/40	14/65	22/86	20/89	29/103	20/76	23/85	16/70	30/115	56/236	56/210	
Total	Positive (%)	22.6	27.5	21.5	25.6	22.4	28.1	26.3	27.0	22.8	26.0	23.7	26.6	
	95% CI	15.1, 31.8	14.6,43.9	12.3,33.5	16.8,36.1	14.3,32.6	19.7,37.9	16.9,37.7	18.0,37.8	13.7,34.4	18.3,35.1	18.5,29.7	20.8,33.2	

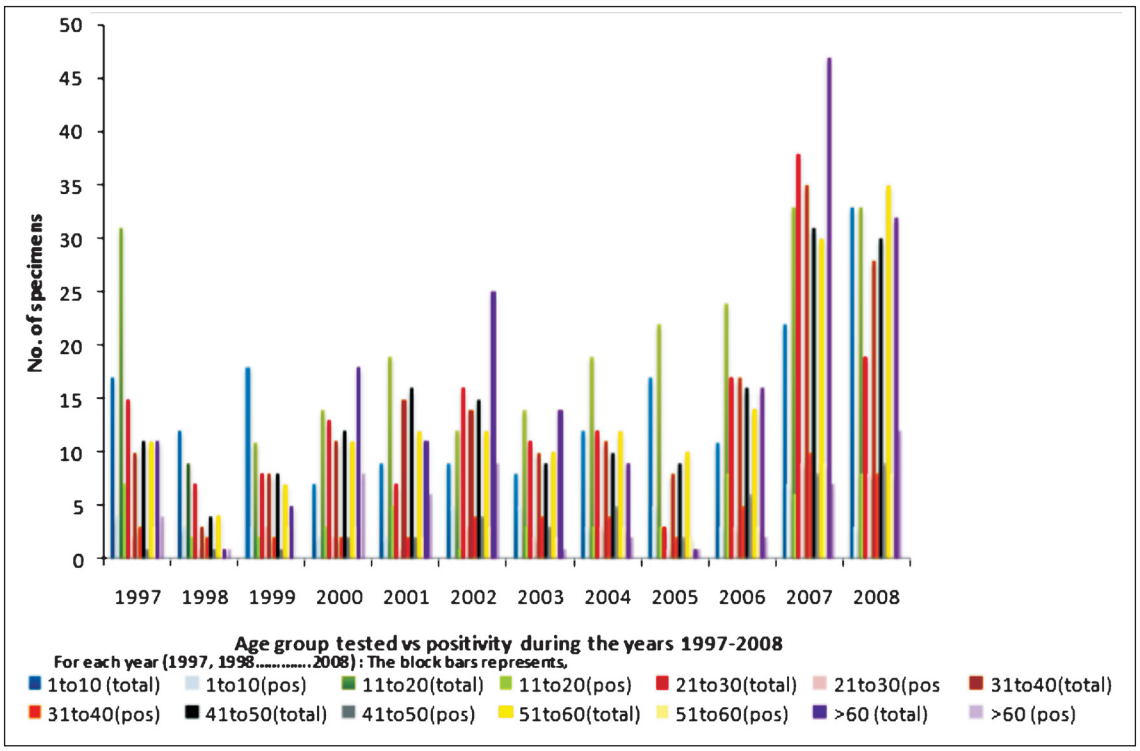

Fig. 2. Number of specimens in different age group and *Chlamydia* antigen positivity during the years 1997-2008.

Patients diagnosed with allergic, acute or follicular conjunctivitis showed significantly (P<0.01) higher positivity (33.4%) than patients labelled as trachoma (27.8%), kerato-conjunctivitis (10.6%) and other eye infections mimicking trachoma (18.9%) (Table III).

Discussion

Success of the global alliance for elimination of trachoma by 2020, (GET2020) initiated by WHO lies in the stringency and outcome of local efforts to contain the disease[2,3]. Any such initiative will have the desired impact only if reliable data pertaining to the prevalence of the disease in an 'erstwhile endemic area' is generated periodically; in which clinical, laboratory and epidemiological accuracy play important roles[1,4,5].

Trachoma prevalence in India has been reported to be varying between 0.5 to 80 per cent, according to studies[6,9-13] conducted across various centers. Community studies were conducted to find out the true prevalence of *C. trachomatis* infection using laboratory support in the known hyperendemic belt of northern India by the Trachoma Study Group in 1998 (Uttar Pradesh)[9] and later in 2007-2008 (Haryana)[10,11]. Hence, the data generated during the present hospital based study for 12 years could be useful and indicative of the trend of *C. trachomatis* eye infection in the region during these twelve years. Clinical diagnosis alone has often been misleading for various reason, like concurrent infections, non detection of *C. trachomatis* in clinically active patients and clinical positivity even after the complete antibiotic therapy because of continuing inflammation[14,15,17].

In the present study, *Chlamydia* antigen was detected in 27.8 per cent of patients diagnosed as trachoma, in 33.4 per cent of patients clinically

Table III. *C. trachomatis* antigen positivity in patients with different clinical diagnosis during 1997-2008

Clinical diagnosis	Total number of patients	*C. trachomatis* antigen positive (%)
Trachoma	485	135 (27.8)
Follicular, acute or allergic conjunctivitis	296	99 (33.4)
Kerato conjunctivitis	94	10 (10.6)*+
Other eye infections/ manifestations	406	77 (18.9)*+
Total	1281	321 (25.05)

*P<0.05 compared to trachoma; +P<0.05 as compared to folliculwar conjunctivitis

diagnosed as follicular/acute or allergic conjunctivitis and in 18.9 and 10.6 per cent in patients with other eye infections and kerato-conjunctivis, respectively. The laboratory testing provided definitive information about chlamydial infection in more patients than clinical examination alone. In our previous hospital based study in the last decade, in a separate small group of patients of chronic conjunctivitis, *Chlamydia* antigen could be detected in 38 per cent patients[18]. However, much lower rates of *C. trachoamtis* antigen detection was reported from hospital based studies from south India[12,13].

Rapid antigen detection by immunoflourescence assay was used in this study which is relatively quicker and easier to perform, and more affordable than PCR assay, and is time tested in other laboratories[19-21]. Although there has been reports on a decline in number of cases with *C. trachomatis* eye infections[13], we found antigen positivity above 21 per cent in all age groups studied. This positivity rate was higher as compared to the findings from community studies[9,10], as this was a hospital based study where patients actively sought medical assistance and thus might not be true reflection of increased prevalence in community. There is a possibility that the patients might have clinical trachoma without having true infection, as was suggested by well established studies from other parts of the world[14,15]. An Australian study found that only in 17 per cent of clinically active cases could *C. trachomatis* DNA be detected[22]. In a community study in Nepal[14] with 6 per cent clinical activity, *C. trachomatis* DNA could be detected in none of the subjects. Our study suggests that *C. trachomatis* eye infection is still continuing in the community, compelling patients for hospital visit. Previous reports indicated increased infectious load with decreasing endemicity and disease severity for trachoma[21,23]. At the same time clinical overdiagnosis resulting in projection of unusually higher prevalence rates has always remained a point of criticism[24]. Therefore, it has become necessary to confirm the clinical diagnosis using one or more duly validated and affordable laboratory tests[15,25]. Several researchers have suggested the use of nucleic-acid based assays including quantitative real-time PCR[26] including pooling of samples[27] to augment clinical assessment. Keeping in view the cost and technical expertise involved, the current generation gene detection assays may be used only for the diagnosis of referred cases or for the purpose of molecular subtyping of circulating strains whereas methods like rapid antigen detection using immuno-fluorescence may be preferred for epidemiological surveillance in developing countries.

In conclusion, the present study indicates that *C. trachomatis* eye infection is persisting in northern India, albeit at a lower level. However, a large population-based nationwide study is needed to identify the exact epidemiology of this infection.

Acknowledgment

This study was supported by Departmental Funding from Dr R.P. Centre for Ophthalmic Sciences, New Delhi. Authors thank all the clinical faculty members and resident doctors of Dr R.P. Centre for Ophthalmic Sciences for sending the clinical specimens for investigations.

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