

Chlamydial eye infections: Current perspectives

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Chlamydia trachomatis, an obligate intraocular bacteria causing trachoma, adult and neonatal inclusion conjunctivitis, was the leading cause of blindness in the last century worldwide. Improvement in socioeconomic and living conditions, availability of antibiotics, and introduction of National Trachoma Control Programmes reduced the prevalence in developed countries, but it persisted in resource-poor settings of Africa and Asia, including India. In 2016, as per the WHO report, trachoma is restricted to 42 countries, causing blindness/visual impairment in ~1.9 million people. India is one of the five countries with nearly half of total active trachoma patients. Introduction of Global Elimination of Trachoma 2020 program by the WHO, using SAFE strategy (surgery for trachomatous trichiasis; Antibiotics for *C. trachomatis*; Facial cleanliness; and environmental improvement) greatly reduced the prevalence, but trachoma still persists in India. Global increase in the reproductive tract infection by *C. trachomatis* urogenital serotypes (D-K) has led to concurrent increase in *C. trachomatis* eye infections. Therefore, kerato eye infections due to chlamydial infections continue to be seen in hospitals. Over the years, there have been advances in laboratory diagnostics, in understanding the pathogenesis, tissue tropism, *C. trachomatis* genomics, and treatment modalities. Due attention and research is still needed for the study of *C. trachomatis* eye infections.

Key words: *Chlamydia trachomatis*, chlamydial eye infection, Global Elimination of Trachoma 2020, inclusion conjunctivitis, SAFE, trachoma

Infections continue to be the most common cause of ocular morbidity and preventable blindness in the underdeveloped and developing countries, accounting for a substantial proportion of all hospital visits.^[1] Historically, trachoma was the most common blinding eye infections in major parts of the world including India. After industrial revolution, general improvements eradicated trachoma from the Western developed world; it continued to persist in Africa, Asia pockets of Australia, and Central and South America.^[2,3] After the adoption of Global Elimination of Trachoma (GET) 2020 program by the WHO in 1996 for trachoma eradication, the prevalence has substantially reduced in major parts of the world; however, as per the WHO report of 2016, the disease persists in 42 countries including India.^[4] In the meantime, the global increase in the occurrence of Chlamydial urogenital infections had increased occurrences of *Chlamydia trachomatis* neonatal and adult chlamydial inclusion conjunctivitis.^[5] In recent years, some advances in understanding the pathophysiology of chlamydial eye infections and tissue tropism have been reported.^[5] Advances in genomics have also promoted genome-wide studies in *C. trachomatis*.^[5]

Causative Agents and Pathogenesis

C. trachomatis belonging to family Chlamydiaceae, a Gram-negative obligate intracellular bacteria is the causative agent of trachoma and inclusion conjunctivitis and

urogenital infections.^[1] *C. trachomatis* has a single chromosome of ~1 Mbp that codes for 875 genes and multiple copies of endogenous plasmids functioning as virulence factors.^[5,6] *C. trachomatis* is biphasic, consists of metabolically active, larger (~1000 nm) reticulate bodies and smaller (~300 nm) resistant and metabolically inert elementary bodies which functions as the infective form.^[7,8] The organism completes its life cycle in conjunctival epithelial cells within 48–72 h with the release of 200 infective EB.^[8] The infected host cells are characterized by the presence of a chlamydial inclusion in the perinuclear region.

The infection evokes an intense mixed inflammatory response in the conjunctiva initiated by the cytokines and interferon released by the infected cells.^[5] Initially, the neutrophils infiltrate the conjunctiva followed by lymphocytes, macrophages, plasma cells, and eosinophils. This diffuse infiltration is accompanied by the presence of intermittent follicles (B cells surrounded by T cells).^[9] Innate proinflammatory immune response by the infected epithelial cells or T-cell response to repeated chlamydial infections leads to trachomatous scarring (TS), in which loose Type I stromal collagen of conjunctiva is replaced by compact Type V collagen.^[10-12] Eventually, goblet cell numbers reduce and epithelial cells are thinned out.^[5]

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Chlamydia trachomatis Serotypes

C. trachomatis have 19 different serovars, belonging to trachoma biovar containing serovars A-K and the lymphogranuloma venereum (LGV) biovar with serovars L1, L2, L2a, and L3.^[8] Endemic trachoma is caused by ocular serotypes of *C. trachomatis* (A, B, Ba, and C). Neonatal conjunctivitis and adult inclusion conjunctivitis are caused by *C. trachomatis* serotype D-K. Neonatal conjunctivitis and adult inclusion conjunctivitis are caused by *C. trachomatis* serotype D-K.^[8]

The major outer membrane protein (MOMP) accounts for about 60% of the total surface protein. Variations in MOMP epitopes determine the type of serovar and may be an important target for the immune response.^[8] Extensive recombination occurs between different strains, so genotyping based on the *pmp* gene nucleotide sequence is not always reliable. Even though basis for the tissue tropism of the serotypes is not yet fully known, but the ocular serotypes lack the capacity to synthesize tryptophan and polymorphism is observed in their *tarp* and *pmp* genes.^[5]

Transmission

C. trachomatis spread by either direct contact or indirect contacts through fomites, hands, bedding, flies, and contaminated towels, etc.^[13,14] Family and schools are the main settings for the transmission.^[14] In healthy individuals, immune system has sufficient efficiency to clear a single episode of chlamydial infection; however, after multiple episodes of infections, it fails to combat the infection. In the endemic communities, reacquisition of the chlamydial infection occurs frequently within a short period of time that results in multiple infections, inflammation, and visual complications.^[14]

Clinical Features

Trachoma usually affects both the eyes and symptoms include itching, irritation, discharge, swelling of eyelids, photophobia, and pain. During the initial stage, follicles appear in the upper tarsal conjunctiva which contains white blood cells followed by papillae.^[15] Repeated infections lead to scarring of the conjunctiva, ranging from a few linear or stellate scars to thick distorting bands of fibrosis which appear as white lines with split lamp.^[3,8] The scar tissue contracts which results in entropion and trichiasis leading to corneal opacification and ultimately blindness.^[3,8] The WHO parameters for the stages of trachoma are trachomatous inflammation-follicular; trachomatous inflammation-intense; TS; trachomatous trichiasis (TT); and corneal opacity (CO).^[8]

Symptoms of neonatal inclusion conjunctivitis, namely, ocular discharge, conjunctival congestion, and swollen eyelids appear within the first 15 days of birth.^[8,16] Symptoms of the adult inclusion conjunctivitis include unilateral mucopurulent discharge, foreign body sensation, redness, tearing, photophobia, hyperemia of tarsal conjunctiva rather than bulbar conjunctiva, and appearance of swollen lymph nodes around the eyes.^[3,8]

Epidemiology

Global scenario

In 1990, the WHO reported that 146 million individuals across the globe had active trachoma, 10 million were in need of

surgery, and 8 million were blind due to trachoma.^[13,15,17] In 1995, about 15.5% of the total blindness across the world were due to trachoma and it was the second major cause of global blindness.^[18,19] Therefore, in 1995, the World Health Assembly constituted the Global Alliance for the Elimination of Blinding Trachoma by the year 2020 (GET 2020) by implementation of the SAFE strategy (surgery for TT; antibiotics for *C. trachomatis*; facial cleanliness; and environmental improvement) with an aim to eliminate trachoma by the year 2020.^[4,20] With the implementation of SAFE strategy, trachoma has decreased in significance as a major cause of blindness.^[21-23] In 2002 and 2003, 84 million people across the world were suffering from active trachoma and ~1.3 million people were blind from trachoma. In 2002, ~3.6% of the total visual impairment was due to trachoma, which was the fourth major cause of blindness.^[21-25] In 2005, as per the WHO report, ~60 million people were suffering from trachoma and in 2008, 40 million people were suffering from active trachoma (WHO report, 2005 and 2008).

A large number of population in densely populated countries such as India, Nepal, China, and within Africa continent are still suffering from trachoma.^[26,27] In 2004, 8% of Nepalese population (2.16 million) had active trachoma prevalence, which was reduced by 90% in 2012 with implementation of SAFE strategy by the National Trachoma Programme.^[28] In 2013, the WHO reported that trachoma was a major public health problem in 53 socioeconomically underdeveloped countries of the world in Africa, Central and South America, Asia, Australia, and the Middle East.^[29] As per the WHO alliance of GET 2020 database 2016, 200 million people are living in trachoma endemic area and are responsible for blindness or visual impairment of ~1.9 million people. In these areas, active trachoma is common among preschool children, with high prevalence rates of 60%–90%. Seven countries (China, Gambia, Ghana, the Islamic Republic of Iran, Morocco, Myanmar, and Oman) had submitted reports of achieving 100% elimination goals of trachoma, which was a major milestone in the campaign to eliminate chlamydial infections of the eyes (WHO report 2016).

Indian scenario

India is recognized as one of the major endemic zones of chlamydial eye infection. Currently, it is one of the five countries having about half of the total active trachoma cases of the world.^[2] Drier parts of Northern India were a major endemic focus for chlamydial eye infections.^[3,30] Even today, chlamydial eye infections are more common in city slums and crowded areas mostly inhabited by the underprivileged sections of the society due to lack of adequate water supply, poor personal hygiene, inadequate waste disposal, etc.^[31]

Before 1947, exact trachoma prevalence information was not available. Trachoma control pilot project was launched by the Government of India in 1953 with assistance from the WHO and the United Nations International Children's Emergency Fund. As per their report, trachoma was hyperendemic in Northern states with the prevalences of Punjab (79.1%), Uttar Pradesh (68%), Rajasthan (74.2%), and Gujarat (56%). The Northeastern and Southern states had very low prevalence (0.5%–8%).^[32,33] Based on these results, the National Trachoma Control Program (NTCP) was launched in 1963 in the states with trachoma prevalence > 50%, with implementation of health education and mass antibiotic treatment of children

below 10 years.^[34] In 1966, NTCP was integrated into general health services. In 1976, it became a part of the National Program for Control and Prevention of Blindness and was extended to 26 states.^[35] Corrective measures of health education, mass antibiotic treatment (tetracycline), and surgery for trichiasis were introduced in these states; trachoma prevalence was reduced.^[33] In 1982, a survey on blindness by the National Survey Organization of India reported 3.47 million total blind people and 20% of the blindness was due to trachoma.^[36]

From 1986 to 1989, a national survey for evaluation of trachoma control activities was undertaken by the Government of India, through Dr. Rajendra Prasad Centre for Ophthalmic Sciences with the WHO support. As per its report, the active trachoma prevalence was reduced to 10.6%–13.8% in the 4 previously endemic Northern states (Punjab [13.4%], Uttar Pradesh [10.6%], Rajasthan [13.8%], and Gujarat [7.1%]).^[33] In all other states, the active trachoma prevalence was very low (0.2%–2.8%).^[33]

Still there were pockets of high prevalence in Northern India including Delhi.^[33] In 1989–1990 in a Delhi community school from a poor locality, 96 of the 108 (89%) school children (9–12 years) had clinical signs of active trachoma.^[37] In a laboratory-based field study in hyperendemic areas of Uttar Pradesh (Bulandshahr and Dehradun districts), in 1998, the prevalence rate of 8.5% active trachoma among 837 children in the age-group of 1–10 years was reported.^[38] In small studies in Delhi in 1999 and 2004 suggested, trachoma was common in Delhi school children^[39,40] In 2005, another laboratory-based study was conducted in rural areas of Mewat region of Haryana; in 1000 children (age: 1–9 years), the prevalence of active trachoma was 4% and in 1000 adult females (500 between 15 and 30 years and the rest over 30 years), the prevalences were of TS 26.4%, TT 5.4%, and trachomatous CO 3.2%.^[38,41]

A survey was undertaken in two villages of Great Nicobar Island in 1991–1992, by Dr. Rajendra Prasad Centre for Ophthalmic Sciences reported 89% prevalence of active trachoma in children and serum antibodies against *C. trachomatis* in 42% of the patients. *C. trachomatis* antigen was detected in 50% of the patients.^[42]

In 2010, clinical screening of 516 children (1–9 years) in Car Nicobar Island revealed 50.5% children had active trachoma and 15% of children had noticeable unclean faces. An average of 7.5% of the 7277 adults screened (>15 years of age) had evidence of TT.^[43] After three rounds of azithromycin therapy, in 2013, a laboratory-based study was undertaken in the same population and the prevalence of active trachoma in children aged 1–9 years was 6.8% and the prevalence of TT was 3.9%.^[44] In 2015, a clinical study from central India in 110 children revealed, 16.36% had follicular trachoma while 10.91% had trachoma intense.^[45]

Chlamydial Infections in Hospital Attendees

Hospital attendance due to clinically suspected chlamydial eye infection is common in Northern India. A 12-year-old (1997–2008) study on *C. trachomatis* detection in follicular conjunctivitis patients reported antigen positivity of 22%–28% in the conjunctival smear with a male predominance.^[46] In 1996–1997, in a small study in fifty patients with chronic

conjunctivitis, *C. trachomatis* antigen was detected in 38% of the patients.^[47] In another study in 70 neonatal conjunctivitis cases in a pediatric ward, 24% of the neonates were *C. trachomatis* antigen positive in conjunctival smears.^[48] For the last 5 years (2009–2015), we had screened 626 patients with overt clinical signs of *C. trachomatis* eye infections for *C. trachomatis* antigen and observed an antigen positive rate of 49.2% by monoclonal-based direct immunofluorescence assay (DFA).

A laboratory-based study from Chennai, in 1995, reported that from 127 conjunctivitis patients, 44 (34.64%) were *C. trachomatis* culture positive, whereas 19 (14.9%) had clinical suspicion.^[49] From 1990 to 1998, in 1061 conjunctivitis patients attending a Chennai eye hospital, 20.9% had laboratory evidence of *C. trachomatis* infections.^[50] In 2002, same laboratory reported only 16 (4.9%) of 328 conjunctivitis patients were *C. trachomatis* positive by polymerase chain reaction (PCR) assay.^[51] In a clinicoepidemiological study from the Regional Institute of Ophthalmology and Sitapur Eye Hospital Clinic, Uttar Pradesh; in 2014, it was reported that the prevalence of active trachoma was 64% and the prevalence of chronic trachoma was 36.7%.^[52]

From an eye hospital in Florida, USA, in 1986–2014 period, *C. trachomatis* positivity in follicular conjunctivitis was 13.4%–5.8% with a median of 10.5%. Rate of prevalence was more in the earlier years (1986–1989: 13.4%) which declined slowly, and a sharp decline was noticed in the recent years (2010–2014: 5.8%).^[53]

Laboratory Diagnosis

Follicular conjunctivitis by other agents, especially adenoviruses, can be misdiagnosed as chlamydial infections. The earliest and easiest method of laboratory diagnosis was by direct detection of inclusion bodies (Halberstaedter-Prowazek bodies) with Giemsa staining of conjunctival smears.^[45,46] The test has low sensitivity and specificity.^[54,55] Antigen detection assays such as DFA with monoclonal antibodies have sensitivity of 85%–90% and enzyme immunoassay (EIA) have sensitivity of 85%–90%.^[48,54-57] Both are used widely in routine. Tissue culture isolation of *C. trachomatis* in Hella cells or McCoy cells, etc., is the most specific method and considered the “gold standard.” However, sensitivity is low (up to 40%).^[55] *C. trachomatis* inclusions are detected either by Giemsa staining or immunofluorescence assay after 48–72 h of incubation.^[54,55] The cells require prior treatment with growth retarding agents, namely, cyclohexidine/5-iodo-2-deoxyuridine, mitomycin C, or irradiation. Due to low sensitivity of “gold standard test,” there was a suggestion for an “extended gold standard test” comprising tissue culture isolation with an antigen detection assay for *Chlamydia* laboratory diagnosis. Nucleic acid amplification test (NAAT) test like PCR assay is the most sensitive and specific technique for *C. trachomatis* detection.^[54,58-61] In a study using PCR assay and antigen detection by DFA in 178 patients with follicular conjunctivitis, *C. trachomatis* was detected in 53.37% samples by PCR assay and in 38.76% patients by DFA.^[62] NAAT tests are still not used for routine laboratory practices in resource-poor countries due to cost and lack of expertise. Recently, quantitative real-time PCR has been used to measure the load of *C. trachomatis* in patient samples.^[63] Other nucleic acid detection/amplification tests such as gene probe assay, loop-mediated isothermal amplification

assay, ligase chain reaction assay, and strand displacement amplification have been tried or are under development for *C. trachomatis* detection, but none are used routinely.^[64]

Role of Serology in Laboratory Diagnosis

In the 1970s, after micro-immunofluorescence assay for chlamydial antibody detection using purified EB was developed, a number of studies were carried out to estimate the seroprevalence of chlamydial infections throughout the world.^[65-67] Infection by any one serotype of *C. trachomatis* evokes cross-reactive antibody response against all the serotypes.^[67] The antibodies persist for a long time; therefore, serology has limited role in diagnosis of acute/active chlamydial infections. However, serology may be helpful in the diagnosis of chronic and invasive infections such as LGV.^[64] Later, it was observed that the antibodies against *Chlamydomphila pneumoniae* are highly cross-reactive with *C. trachomatis* antibodies. Therefore, it became clear that the exact prevalence of *C. trachomatis* infection cannot be easily determined from antibody detection.^[66]

In a 1988–1989 study in a Delhi school, most of the 96 clinically active trachoma patients had genus specific antichlamydial antibodies.^[37] By studying type-specific antibody response by microimmunofluorescence (MIF) assay against serotypes A, B, Ba, C, and D in 32 of these inclusion positive children, serotype A was concluded as the causative serotype.^[68] In 1992–1993, in a hospital-based study in eighty follicular conjunctivitis patients, *Chlamydia* antibodies were detected in 67.5% patients by MIF assay and 70% patients with EIA assay.^[69] A study was done to detect *C. trachomatis* antibodies in 182 neonates (4.4% of them were positive) and 216 children (1 month–5 years), of whom 1.4% were positive for *C. trachomatis* antibodies.^[16] To find out the influence of *C. pneumonia* antibodies in *Chlamydia* serology, a study was conducted on 844 healthy blood donors and reported that chlamydial antibody prevalence was 55.69%. Of these, 42.77% had antibodies against *C. pneumoniae* and 12.5% against *C. trachomatis*.^[70]

Till now, methods to detect *C. trachomatis* specific antibodies are not available or rarely available. More recently, there is revival in use of serology in *C. trachomatis* eye infections.^[71] In recent times, the diagnostic performance of *Chlamydia* antibody detection was improved using species-specific proteins or peptides. Immunogenic proteins of *C. trachomatis* identified by two-dimensional polyacrylamide gel electrophoresis were used with analogous proteins of *C. pneumoniae* and *Chlamydomphila psittaci* to allow differential evaluation of *Chlamydia* antibody reactivity.^[72] In a trachoma surveillance based on serology for the 12-year cohort of Kahe Mpya, Tanzania, it was reported that the prevalence of trachoma was 6.5%, whereas only 3.5% were seropositive.^[71]

Conclusions

Chlamydial eye infections have declined to a significant level in the previously endemic countries worldwide due to the improvements in the living standards, availability of primary health care, and constant effort of the health workers and Governments. In GET 2020 program, trachoma eradication is defined as the prevalence of active proven trachoma below 5% level.^[4,8] However, in highly populous countries such as India

and China, even a low prevalence will translate into a huge number of persons with active trachoma, who can potentially transmit the infections to other people; hence, they cannot be neglected. Moreover, in large countries such as India, it may not be possible to estimate the exact trachoma prevalence in remote areas. It has already been reported that, after mass azithromycin therapy for eradication, trachoma cases are recurring after a few years.^[44] Reproductive tract infections by *C. trachomatis* are increasing in developing countries including India, so simultaneous chlamydial eye infections are being observed in hospital attendees.

For many socioeconomically backward communities of the world, it may take many decades for improvements in the living standards and hygiene, so as to eliminate chlamydial infections completely. Therefore, the topic is still relevant and it is necessary to keep a vigil on *C. trachomatis* eye infections.

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Conflicts of interest

There are no conflicts of interest.

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