A hospital based study on the prevalence of conjunctivitis due to \textit{Chlamydia trachomatis}

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\textit{Background & objectives}: Very few studies have been done in India to determine the prevalence of \textit{Chlamydia trachomatis} causing conjunctivitis using polymerase chain reaction (PCR) methods. Hence the prevalence of primary conjunctivitis due to \textit{C. trachomatis} among individuals attending ophthalmic hospitals in Chennai was determined and compared with our earlier results.

\textit{Methods}: A total of 328 conjunctival swabs from 255 (both eyes 73 and one eye 182) patients were investigated by fluorescent antibody test (FAT) on direct smears, culture and PCRs for cryptic plasmid and major outer membrane protein (MOMP1) gene of \textit{C. trachomatis}. An infant with ophthalmia neonatorum was also included.

\textit{Results}: Among these 328 specimens processed, 16 (4.9\%) from 12 (4.7\%) patients were positive by cryptic plasmid PCR. Among these, 3 from 2 patients were positive by FAT (direct smear), culture and PCR for MOMP1 gene. Both eyes of the infant with ophthalmia neonatorum were positive by all the methods. The sensitivity of FAT and culture (18.8\%) was lower compared to PCR.

\textit{Interpretation & conclusion}: A significant decrease in the prevalence of adult chlamydial conjunctivitis has occurred in the 10-year period among patients reporting to the ophthalmic hospitals in Chennai. PCR using cryptic plasmid primers was found to be the most sensitive method to detect \textit{C. trachomatis} in patients with conjunctivitis.

\textit{Key words} \textit{Chlamydia trachomatis} - chlamydial conjunctivitis - polymerase chain reaction (PCR) - prevalence

\textit{Chlamydia trachomatis}, an obligate intracellular bacterium is the causative agent of trachoma, inclusion conjunctivitis and lymphogranuloma venereum. Trachoma, a blinding, endemic disease which spreads from eye to eye is associated with infections by serovars A, B, Ba and C of the bacterium. Adult inclusion conjunctivitis is usually associated with genital infection which is a sexually transmitted disease caused by serovars D to K of \textit{C. trachomatis}. The newborn infant acquires conjunctivitis (ophthalmia neonatorum) due to these strains during birth from the infected cervix of the mother\textsuperscript{1}. In the earlier hospital-based studies on primary conjunctivitis, we had shown the prevalence of \textit{C. trachomatis} infection to be 17 per cent in 1990 and 34.6 per cent in 1991-1992\textsuperscript{2,3}. In these studies, chlamydial conjunctivitis was diagnosed by detection of the antigen using immunoperoxidase test or fluorescent antibody test (FAT) and by isolation of the organism in McCoy cell cultures. Molecular techniques like polymerase chain reaction (PCR) and ligase chain reaction have recently been introduced to detect the bacterium in clinical specimens\textsuperscript{4-7}. In the present study, the current prevalence of Chlamydial conjunctivitis was determined for comparison with our earlier results\textsuperscript{2,3}. In addition to the conventional methods used in the earlier studies, PCR was also applied.

\textit{Material & Methods}
Patients & specimens: Consecutive adult patients with primary conjunctivitis attending the outpatient departments of the Regional Government Ophthalmic Hospital and Sankara Nethralaya Ophthalmic Hospital, Chennai were included in this study during a period of 15 months between November 2000 to January 2002. Conjunctival swabs were collected from patients with primary conjunctivitis which was defined for the purpose of the study as a primary inflammation of the conjunctiva not related to any adnexal disease or intraocular inflammation or trauma or resulting from application of topical medication. A total of 328 conjunctival swabs were collected from 255 (both eyes - 73 and one eye only - 182) patients. Conjunctival swab from a 10 day-old infant with ophthalmia neonatorum were also included in the study.

Conjunctival swabs placed in 2M-sucrose phosphate (2SP) buffer were stored at -80°C until processed for culture and DNA extraction for PCR. Culture and PCR were performed within 48 h of collection of the specimen. Smears of the conjunctival specimens were prepared using another sterile swab for the direct antigen detection of *C. trachomatis*.

**Fluorescent antibody test (FAT) on direct smear & culture for C. trachomatis:** Smears of the conjunctival specimens for direct antigen detection of *C. trachomatis* were stained by FAT.

**Cultivation of C. trachomatis** was done in shell vials with McCoy cell line (the cell line was obtained from National Centre for Cell Science, Pune, India and maintained in our tissue culture laboratory) pretreated with 1 µg/ml concentration of cycloheximide (HiMedia, India). As a routine, controls for FAT and culture were done at frequent intervals in the laboratory to verify and confirm that these tests give good results by inoculating McCoy cell cultures with the least amounts of standard strains (*C. trachomatis* serotype Ba ATCC-VR 347, procured from CDC, Atlanta) and staining the same by FAT.

**Polymerase chain reaction:**

(i) DNA extraction — DNA extraction was done by guanidine thiocyanate method (GIC).

(ii) Primers & thermal cycle profile for PCR — Two sets of primers targeted against the cryptic plasmid DNA and major outer membrane (MOMP 1) protein gene of *C. trachomatis* were used.

Optimization of PCR cryptic plasmid was done with DNA extracted from the cell culture harvest of *C. trachomatis* Ba (ATCC-VR 347) grown in McCoy cell culture. Amplification was carried out in a 50 µl volume using 10p moles of primers derived from the sequence of the cryptic plasmid of *C. trachomatis*, containing 1.5 units of Taq polymerase in 10X PCR buffer, 200mM dNTPs, and 10 picomoles of cryptic plasmid primers. The sequence of the primers are:

**Forward primer:** 5’ TTC TCA TCA TAA AAA CAT ATT CAT AGT AT-3’

**Reverse primer:** 5’ CTG ACT GTG AGA ATA TAT CAT AAA TAG AC-3’.

Optimization of PCR using primers specific for sequences in the major outer membrane protein (MOMP 1) gene of *C. trachomatis* was done. The sequence of the MOMP 1 primers are:

**Forward primer:** CT1: 5’ CCT GTG GGG AAT CCT GCT GAA-3’

**Reverse primer:** CT2 5’GTC GAA AAC AAA GTC ACC ATA GTA-3.’

Optimized product of 370 base pairs was obtained after amplification.
primers yielded an amplified product of 144 base pairs. DNA was amplified in 5μl volume containing 200mM dNTPs, 10X PCR buffer, 10 picomoles of primers and 2 units of Taq polymerase. The reaction profile consisted of initial denaturation at 94°C for 4 min followed by denaturation at 95°C for 1 min, annealing at 45°C for 2 min, and extension at 72°C for 2 min for a total of 49 cycles followed by a final extension at 72°C for 4 min. DNA extraction, amplification of DNA and analysis of the amplified products were carried out in separate rooms.

(iii) Visualization of the PCR amplified products — Amplified products were visualized on an agarose gel incorporating 2μg/ml ethidium bromide and were visualized by UV fluorescence transilluminator (Pharmacia, Biotech, USA).

Fig. Agarose gel electrophoretogram showing plasmid amplified products of Chlamydia trachomatis. Lane 1 represents negative (buffer) control; Lane 2 represents extraction control; Lane 3 represents conjunctival swab negative for C. trachomatis; Lanes 4 & 5 represent conjunctival swabs positive for C. trachomatis; Lanes 6 & 7 represent conjunctival swabs negative for C. trachomatis; Lane 8 represents positive control DNA (C. trachomatis - ATCC -VR 347); Lane 9 represents molecular weight marker (φX DNA/ Hinf I digest).

(iv) Sensitivity & specificity of primers — The sensitivity of the primers was estimated by counting the number of elementary bodies present in the culture harvest of C. trachomatis Ba (ATCC-VR 347). Then the extracted DNA was serially 10-fold diluted, amplified so that the number of elementary bodies that could have been present in each dilution, were calculated.

Specificity of the primers was tested against DNA extracted from adenovirus type 2 (ATCC-VR-846), herpes simplex virus (ATCC 733 VR), Staphylococcus aureus (ATCC 25293), Haemophilus influenzae (ATCC 35056), Mycobacterium tuberculosis (H37Rv) and laboratory isolates of Streptococcus pyogenes, Klebsiella pneumoniae, Propionibacterium acne, and DNA extracted from human blood.

In this study PCR was considered as the gold standard and the specificity and sensitivity of culture and FAT was calculated accordingly.

Results

Specificity and sensitivity of PCR for cryptic plasmid & MOMP 1 genome of C. trachomatis: PCR tests using cryptic plasmid and MOMP1 primers showed the presence of 370 bp and 144 bp amplified products of C. trachomatis DNA respectively with no amplified products from DNA extracted from non-C. trachomatis organisms and human DNA indicating their absolute specificity. Regarding the sensitivity, the cryptic plasmid primers detected up to 2ng of C. trachomatis DNA that corresponded to 10 elementary bodies of C. trachomatis. The MOMP1 primers could detect up to 6 ng of C. trachomatis DNA corresponding to 30 elementary bodies.
showed low sensitivity. Among the 328 specimens from 255 patients processed, 16 (4.9%) specimens from 12 (4.7%) patients were positive by plasmid PCR (Fig.). Among these 16 specimens, 3 (0.91%) from 2 patients were positive by FAT, culture and PCR for MOMP1 gene. These included a case of ophthalmia neonatorum in whom both eyes were positive for *C. trachomatis*.

In this study PCR was considered as the gold standard and the sensitivity of FAT and culture was determined to be lower (18.8%). The specificity of the FAT and culture was determined to be 100 per cent.

**Discussion**

The results of this study clearly demonstrated a significant reduction in the number of adult patients with primary conjunctivitis due to *C. trachomatis* infection reporting to the major ophthalmic hospitals in Chennai compared to the prevalence reported by us 10 yr earlier (1991-92). In the earlier study, 127 adult patients were investigated and 44 (34.6%) were found to have culture proven primary chlamydial conjunctivitis. The present study included twice the number of adult primary conjunctivitis patients (254), but only one was culture proven. One case of ophthalmia neonatorum also was culture proven with isolation of *C. trachomatis* from both eyes. The shell vial culture technique in McCoy cell line was verified for its accuracy during the period of study employing controls of cultures of the standard strains of *C. trachomatis*. Our results show that the adult chlamydial conjunctivitis is at present an uncommon disease in patients reporting to the major ophthalmic hospitals in Chennai. Even if the 11 adult patients identified to have chlamydial conjunctivitis among the total 254 adult cases were considered, the prevalence (4.7%) is significantly low compared to 17.0 to 34.6 per cent in our earlier studies. Similar reports on the prevalence of adult chlamydial conjunctivitis at different periods of time in any given geographical area were not available on a literature search. Occasional reports indicate the occurrence of lower rates of adult and neonatal chlamydial conjunctivitis. In a study from Saudi Arabia reported in 1995, only 3 per cent of adult patients with conjunctivitis were positive for chlamydial DNA by PCR. A decrease in the occurrence of chlamydial neonatal conjunctivitis from 4.39 per cent in 1995, to 0.78 per cent in 1998 was reported from Argentina possibly indicating a lowering of the rates of cervical infections in the mothers. Mertz et al have reported the prevalence of *C. trachomatis* infection among black women to have decreased by 19 per cent in a 4 yr period.

Of the 12 cases of *C. trachomatis* conjunctivitis identified in the present study, 10 were identified only by PCR detecting cryptic plasmid of the bacterium. This indicated that viable and cultivable organisms are not present in the clinical specimens. This was confirmed by passing these 10 McCoy cells inoculated specimens thrice in the cell cultures before considering them as negative for *C. trachomatis*. We modified the amplification protocol of Bailey et al to increase the sensitivity of PCR for cryptic plasmid with an initial denaturation at 94°C for 4 min and final extension at 72°C for 4 min. Cryptic plasmid primers were more sensitive than the MOMP1 primers and this could be due to the presence of 7-10 copies of plasmid in the organism. In this study, PCR for MOMP1 was positive only in the specimens with high antigenic load as shown by their positivity by FAT and culture.

Though isolation of the organism in cell culture is considered to be the gold standard, its low sensitivity in detecting *C. trachomatis* precludes its use in routine clinical microbiology. In our study we considered PCR as the gold standard and the sensitivity of FAT and culture was determined to be very low (18.8%). FAT on direct smear is a rapid and specific method but its
limitation is the low sensitivity and in our study it was not helpful in detecting the organism from the specimens when compared to PCR.

We do not have a clear history of ocular medications in most of our patients though they appeared to have used some topicals. We believe that this marked reduction in the prevalence of adult chlamydial conjunctivitis in 10 yr in Chennai might be due to frequent self-medication with antibacterial topical drops for any redness in the eyes because of their availability across the counter in pharmacies. This may also explain the low load or absence of viable *C. trachomatis* in the conjunctival specimens positive by PCR.

In conclusion, the prevalence of adult chlamydial conjunctivitis among patients reporting to the outpatient departments of major ophthalmic hospitals in Chennai is reduced. From our study the prevalence of conjunctivitis due to *C. trachomatis* is determined to be 4.7 per cent. There is a marked reduction in prevalence on comparing with our earlier results\(^2,3\). PCR using primers for detection of cryptic plasmid of *C. trachomatis* should be used to detect this bacterial infection of conjunctiva.

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**References**


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