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ORIGINAL ARTICLE / RESEARCH

Role of *Chlamydia trachomatis* in the Aetiological Profile of Chronic Conjunctivitis in a Tertiary Care Hospital

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ABSTRACT

Background: The aetiological profile of chronic conjunctivitis in North-Indian patients is poorly understood; and clinical assessment remains the diagnostic criteria without supportive laboratory investigations. This study was conducted to find out the presence of *Chlamydia trachomatis* and other bacteria in the conjunctiva of patients with clinically diagnosed chronic conjunctivitis, attending the out-patient department of a tertiary eye-care hospital in Delhi.

Methods: One hundred eyes from 50 patients of chronic conjunctivitis and an equal number of apparently healthy controls were included in the study. Conjunctival swabs were collected from the superior/inferior palpebral conjunctiva and eye-lid margins with a sterile wet cotton swab for chlamydial antigen detection by direct immunofluorescence (DFA), bacterial culture, and cytology.

Results: Patients with chronic conjunctivitis revealed 38% positivity for *C.trachomatis* antigen alone, and 15% for mixed bacterial and chlamydial infections, while in the control group, only 2 eyes were positive for chlamydial antigen, and 19 for other bacterial flora. Among bacterial isolates (28), coagulase –ve staphylococci (21) predominated, followed by coagulase +ve staphylococcus (4), dipheroids (2), and aerobic spore-bearing bacilli (1). In cytology (with geimsa stain), inflammatory response was observed; the combination of polymorphs with lymphocyts (62) outnumbered the combination of polymorphs with eosinophils (37) in the case of chronic conjunctivitis.

Conclusions: Findings of the present study suggest that *C.trachomatis* continues to be the leading cause of chronic conjunctivitis in North India. However, surveillance involving larger groups of patients is warranted to further augment our observation.

Key words: Chronic conjunctivitis, *Chlamydia trachomatis*, direct immunofluorescence assay

Key message:

1. Northern India has been a major endemic focus of trachoma in the past. Though there has been a major reduction in trachoma related blindness in India, hospital records still reflect a substantial number of diagnoses of trachoma.
2. This study highlights that chlamydia is still an important infective aetiological agent in chronic conjunctivitis.
3. Super imposed bacterial infections were also observed in 15% of the cases, and these may aggravate the symptoms.

Introduction

Chronic conjunctivitis is an ocular surface manifestation of diversified aetiology, ranging from microbial infections to allergy, topical medication, irritants, acne rosacea, malignancy, contact lens use, and floppy eyelid syndrome, besides many others [1–5]. It is characterized by conjunctival hyperaemia and/or discharge, usually persisting for over 2-4 weeks. Reports from different geographic regions have varied aetiology. However, microbial origin remains the leading cause, more so, in developing countries with relatively inferior hygienic conditions [6–8]. Limited studies have been carried-out to unveil the aetiology of chronic conjunctivitis in India, but most cases in the country still get symptomatic treatment, without ascertaining the exact cause through appropriate laboratory confirmations [9],[10].

A case control study was therefore, designed to gauge the magnitude of bacterial aetiology with due emphasis on Chlamydia, in out-door patients of chronic conjunctivitis attending a tertiary-care hospital in North-India.

Materials and Methods

Selection of patients

One hundred eyes of 50 consecutive patients of chronic conjunctivitis with complaints of itching, redness, irritation and discharge, reporting to the outpatient department of Dr. R.P. Centre for Ophthalmic Sciences the Centre, were recruited for the study. One hundred healthy eyes of 50 normal individuals were taken as controls. Eyes with chronic conjunctivitis of known aetiology i.e. vernal catarrh, phylectenular conjunctivitis, chemical injuries, topical medications, chronic dacrocystitis, and keratoconjunctivitis sicca, were excluded. The mean age of the cases was 32.25 ± 23.83 (9-56 years, 42 males and 8 females), and that of the controls was 32.5 ± 20.34 (12-53 years, 41 males and 9 females). An informed consent was obtained from all patients. A careful clinical examination, including slit lamp biomicroscopy of the anterior segment of the eye and ocular adnexa, was done.

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Specimen collection and processing

Conjunctival swabs were collected from the superior/ inferior palpebral conjunctiva with sterile cotton swabs, and rolled onto glass slides to make the smear for (I) Gram Stain (II) Giemsa Stain (IIII) *C.trachomatis* antigen detection. Separate conjunctival specimens in sterile cotton wool swabs, were collected and inoculated on blood and chocolate agars for bacterial culture.

While bacterial isolates were identified using recommended biochemical tests [11], *C.trachomatis* antigen was detected using a monoclonal based direct immunofluorescence assay, using the commercial kit (Micro-Trak, Syva, UK) as per the manufacturer's instructions [12]. Briefly, the conjunctival smears were brought to room temperature. The smears were covered with 30µl of the FITC conjugated *C. trachomatis* murine monoclonal antibodies, and incubated for 15 minutes at room temperature. The slides were washed in distilled water, air dried, mounted, and observed under the 100X objective of a fluorescent microscope. (NIKON, Japan). A positive and negative control smear (provided with the kit) was included with the test.

Results

In Giemsa stained smears, conjunctival smears of 94 eyes from cases and 20 eyes from controls showed polymorphonuclear cells (PMN). Mixed inflammatory response of PMN and lymphocytes was seen in 62 eyes from cases, and in 3 eyes in controls. Eosinophilic predominance (allergic response) was seen in 37 eyes in cases, and 4 eyes in controls. Chlamydial Halberstaedter - Prowazek (HP) inclusion bodies were detected in 6 eyes from cases, but in none in control smears [Table/Fig 1].

[Table/Fig 1] Conjunctival cytology (Geimsa stain)

Inflammatory cells	Cases (50) (100 eyes) (in percentage)	Control (50) (100 eyes) (in percentage)	*p* Value
Polymorphs only	94	20	<.01
Polymorphs with lymphocytes preponderance	62	3	<.001
Polymorphs with eosinophils preponderance	37	4	<.001
HP inclusion bodies	6	Nil	<.01

In the immunofluorescence assay, Chlamydia antigen alone was detected in 38 eyes (21 patients; 17 bilateral, 4 unilateral) in the patient group, and in two eyes (unilateral) in the control group (p= < 0.0001). Chlamydial antigen detection along with bacterial culture positivity, was seen in 13 eyes (4 bilateral, 5 unilateral) in the patient group, and in 9 eyes in the control group (p value not significant) ([Table/Fig 2]). Of the Chlamydia antigen positive cases, 82% were above 20 years of age, whereas 18% were below 20 years of age.

[Table/Fig 2] Microorganisms detected in chronic conjunctivitis

Investigation	Cases 50 (100 eyes) (in percentage)	Controls 50 (100 eyes) (in percentage)	p Value
C. trachomatis only (DFA)	38	2	<.001
C. trachomatis (DFA) + bacterial growth	13	9	NS
Only bacterial growth	15	10	NS

NS: not significant.

Bacterial culture was positive in 28 eyes from the patient group and in 19 eyes from the control group (p value not significant) ([Table/Fig 3]). Staphylococcus was the predominant organism. Coagulase negative Staphylococci were isolated in 21 cases, coagulase positive Staphylococci were isolated in 4, Diphtheriods were isolated in 2, and Aerobic Spore Bearing bacilli was isolated in one, from patients of chronic conjunctivitis ([Table/Fig 3]).

[Table/Fig 3] Different bacteria isolated in cases and controls

Bacterial species isolated	Cases (50) (100 eyes) (in percentage)	Control (50) (100 eyes) (in percentage)
Staph. epidermidis	21	18
Staph. aureus	4	Nil
Diphtheroids	2	1
Aerobic spore-bearing bacilli	1	Nil

Discussion

Chronic conjunctivitis affects millions of people across the globe, it and requires substantial medical attention to prevent long term sequlae. Different studies have implicated diversified aetiology, but infections remain one of the main causes, more so in developing countries [1].

A cascade of diagnostic methods including non-invasive exfoliative cytology, Giemsa/ Gram's staining, immunofluorescence, and microbial culture in conjunction with clinical wisdom, have been frequently used to establish the aetiology of chronic conjunctivitis. Cytological examination of the conjunctival scrapings revealed the nature of the inflammatory response. Predominance of neutrophils and lymphocytes usually point to bacterial and viral aetiology, while eosinophilic predominance occurs in allergic conjunctivitis [2],[6–8].

In a study carried out in Wisconsin [6], the aetiological agents in chronic conjunctivitis included chlamydiae (19%), viruses (14%), irritants (10%), allergies (7%, bacteria (7%), contact lens (7%), acne rosacea (3%), and floppy eyelid syndrome 2%. In a cytological examination, Cvenkel and Globocnik [2] found

neutrophils in as high as 37%, eosinophils in about 20%, and lymphocytes in just 13% cases of chronic conjunctivitis. Coagulase negative staphylococcus was the most pre-dominant pathogen (`25%), and chlamydia was implicated in less than 7% cases in their study. On the contrary, our present study revealed ≥ 38% positivity for *C.trachomatis,* and 13% for those mixed with bacterial pathogen. In addition, 15% of them had positivity for bacteria. Previous studies from India and other Asian countries have reported a high incidence of *C.trachomatis* in different forms of conjunctivitis, both in adults and in children [7],[9],[10],[12],[13],[14], using the more sensitive restriction length polymorphism (RFLP) and nested polymerase chain reaction (PCR). Madhavan reported `21% positivity for *C.trachomatis* and adenoviral aetiology in 13.8% in cases of conjunctivitis and retinal inflammation in Southern India [7]. Szymulska and Zagorsk [15] also reported high positivity (≥ 34%) of chlamydia in cases of chronic conjunctivitis, using the same method (DFA) as in the present study.

The high incidence of chlamydial aetiology in this part of India could be attributed to poor hygienic conditions and persistence of oculogenital chlamydial infections in the population, at a much higher rate. With regards to bacterial aetiology, we found a high incidence of coagulase negative staphylococci, but there was no significant difference between the cases and the control group. *Staph. epidermidis* was isolated in 21% and in 18%, in cases and controls respectively. However, superadded bacterial infection might have a role in pathogenesis in chronic conjunctivitis cases, due to Chlamydia.

Our finding is in concordance with most previous reports, although Grabson et al. [16] found very high percentage of coagulase negative Staphylococci isolation from normal and inflamed conjunctiva. Assessment of allergic aetiology in the present study, was based on the observation of eosinophilic response in cytology, which was similar to previous observations [4],[8].

It is clear from the results of the present study and those reported elsewhere, that chlamydia remains an important aetiological agent in clinical cases of chronic conjunctivitis, although secondary bacterial infections may aggravate the disease. Extensive studies in different parts of India, however, are recommended to generate true epidemiological data that can represent a national index.

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