

Chlamydia Trachomatis Infections: A Hidden Cause of Meibomian Gland Dysfunction

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Abstract

Purpose: Meibomian gland dysfunction (MGD) is a common eye problem, which is asymptomatic in the early stage, but if left untreated for a prolonged period can exacerbate to dry eye syndrome. Role of bacteria in the pathophysiology of MGD is always controversial. This pilot study was undertaken to find the association of *Chlamydia trachomatis* apart from the normal biota of the eyelid with MGD in a tertiary care hospital in India.

Methods: A total of 112 MGD patients with follicular conjunctival inflammation with either of the symptoms such as; Meibomitis (22), Dry eye (42), Watering (19), Blepharitis (9), Chalazion/ Hardoleum (20) were referred to the Chlamydial laboratory for further investigation. Conjunctival swabs of both eyes were subjected to direct immunofluorescence assay (DFA) for *C. trachomatis* antigen detection.

Results: Of the 112 MGD patients, 62 (55.30%) were found positive for *C. trachomatis* with DFA, out of which 54(87%) had good clinical response for topical azithromycin (1% eye drop), topical lubricants and warm compresses.

Conclusions: We concluded from this study that, *C. trachomatis* is one of the causes for MGD with follicular conjunctival inflammation and topical azithromycin is a good choice for treatment and improving the signs and symptoms of the infection. As the role of chlamydia in MGD is not clear, this study will disclose an exciting fact about the pathophysiology of the disease, which will in turn improve treatment strategy to some extent to combat with the common but frustrating eye problem.

Keywords: Chlamydia Trachomatis; Meibomian Gland Dysfunction; Blepharitis; Dry Eye; Azithromycin; Direct Fluorescence Antigen DFA

Abbreviations: MGD: Meibomian Gland Dysfunction; OSDI: Ocular Surface Disease Index; MMPs: Matrix Metallo Proteinases; DFA: Direct Immune Fluorescence Assay; FITC: Fluoresceine-Isothiocyanate.

Introduction

Meibomian gland dysfunction (MGD) is a chronic diffuse abnormality of the meibomian glands, which is asymptomatic in the early stage, but if left untreated for a prolonged period can exacerbate to the dry eye syndromes, clinically apparent inflammations and ocular surface diseases. In the early stage, MGD is often overlooked by the Ophthalmologists due to its similar signs and symptoms with other eye infections such as; inflammations, irritation, qualitative/ quantitative changes in the secretion of oil from meibomian glands etc [1,2]. As per some earlier reports, up to 70% of people across the world suffer from MGD and it is the leading cause of dry eye disease [3]. As per ocular surface disease index (OSDI) data, the prevalence of dry eye ranges from 5% to 35% worldwide and 29.25% in India [4]. Till today, clinicians and researchers don't have a complete understanding on the underlying mechanism, epidemiology, pathophysiology, and management of MGD, therefore several studies are being carried out across the world to find the hidden cause and mechanism behind its occurrence.

MGD is a chronic abnormality of the meibomian glands that occurs either due to blockage or infections of the glands, so that they stop secreting enough oil over the ocular surface. As oil is the major component of the tear film, MGD at the later stage leads to the evaporative dry eye syndrome or severe eyelid problem like blepharitis [5]. As the role of several bacteria in the pathophysiology of MGD is always controversial, there are some studies which correlate MGD with some bacterial colonization [6]. Some studies demonstrated the role of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Propioni bacterium* with blepharitis, which indicates the possibility that microbes find an altered eyelid environment in MGD more suitable than the normal eyelid [7,8]. These bacteria may have a direct or indirect effects on the meibomian gland function such as production of toxic bacterial products (direct effect) or effects on homeostasis of the ocular surface, such as secretion of matrix metallo proteinases (MMPs), cytokine balance and macrophage function (indirect effect) [9]. As per the theory is concerned, for an antibiotic to be completely beneficial in the treatment of a disease, it must be effective against the

pathogen most likely to be present in the infected area. Hence detection of the exact pathogen responsible for MGD is very essential for the treatment and management of MGD.

This pilot study was undertaken to know the association of *Chlamydia trachomatis* apart from the normal biota of the eyelid with MGD in a tertiary care hospital in India.

Material and Methods

Patient Selection and Collection of Clinical Specimens

In a retrospective study, conjunctival swabs were collected by the Ophthalmologists (after taking consent from the patients following the ethical guidelines of the Institute) from superior/ inferior palpebral conjunctiva of both the eyes of 112 patients (57 females, 55 males; age group 5–65 Yrs; median age: 32.5 Yrs) clinically suspected of MGD with follicular conjunctival inflammation with signs and symptoms of dryness, meibomitis, blepharitis, watering, redness, foreign body sensation, chalazion/ Hardoleum reporting to the outpatient department of Dr RP. Centre for Ophthalmic Sciences, New Delhi, India. Patients with ocular surgery, contact lens uses, eye disorders affecting the ocular surface, infectious conjunctivitis, history of pre-topical antibiotic treatments or systemic steroids use were excluded from this study. Prior to referring the patients to the *Chlamydia* laboratory, experienced ophthalmologists performed clinical examination using slit lamp biomicroscopy of the anterior segment of the eyes of the 112 patients suspected of having MGD, 22 were with the symptoms of meibomitis, 42 with dry eyes, 19 with watering from eyes, 9 with blepharitis and 20 with chalazion/ hardoleum (Table 1).

Conjunctival swabs were collected with sterile wet cotton swabs and rolled on the clean teflon-coated glass slide to prepare the smear (one specimen from each eye). Slides were prepared in duplicates for each sample collected from the patients following the protocol mentioned above. Slides were air-dried, fixed in cold acetone for 10 minutes and subjected to direct immune fluorescence assay (DFA) for chlamydia antigen detection [10]. As the study was focused on to know the association of only *Chlamydia* with MGD; hence samples were not processed to identify the presence of other bacterial flora (apart from *C. trachomatis*) with this infection.

Age Group of Patients (Yrs)	No. Of Samples
0-10	6
11-20	26
21-30	43
31-40	33
41-50	30
51-60	26
61-70	13
71-80	05
81-90	01

Table 1: Trachomatis antigen positivity in MGD patients.

Direct Immune Fluorescence Assay

Direct immune fluorescence assay (DFA) a CDC approved test was performed with the monoclonal antibody based *C. trachomatis* direct specimen kit (Micro-Trak, USA) following the standard protocol mentioned in the kit [10]. Briefly, conjunctival smears were covered with 30µl of fluoresceine-isothiocyanate (FITC)-conjugated murine monoclonal antibodies to *C. trachomatis* for 30 minutes at 37°C in a humidified chamber. The slides were washed with double distilled water, air-dried, mounted and observed under the fluorescent microscope (Nikon, Japan) with 100X objective. Fixed mammalian cells containing *chlamydia* elementary/reticulate bodies (EB/RB) (provided with the kit) were used as the positive-control while normal uninfected mammalian cells were used as the negative control in this pilot study. *C. trachomatis* positive cases were treated with topical azithromycin antibiotic.

Statistical Analysis

Percentage signifies the antigen positivity. P-value was calculated with Chi-square test (Graph pad software) among the male and female MGD patients. Confidence of Interval was calculated with the formula $CI = \text{Sample mean} \pm 1.96 \times \text{SD}/\text{root of sample size}$ (95% confidence level). Degree of freedom was calculated as sample size - 1.

Results

Of the 112 patients (57 females, 55 males; age 4 - 65 Yr) of MGD with conjunctival follicles, 62 (55.35%) were found positive for *C. trachomatis* antigen with DFA. Mean age of the patients was 33.77 years. Of the 22 patients of having symptoms of meibomitis, 16(72.7%) were found positive, of the 42 patients with symptoms of dry eye 18(42.8%) were found positive, of the 19 patients with symptoms of watering from eyes 13(68.4%) were found positive, of the 9 patients with symptoms of blepharitis

5(55.5%) were found positive and of the 20 patients with chalazion/ hardoleum 10(50%) were found positive for *C. trachomatis* antigen with DFA (Table 1). Of the 55 males 30(54.5%) were found positive and of the 57 females 32(56.1%) were positive for *C. trachomatis* antigen with DFA. Females show slightly higher positivity compared to males (Two tailed P- value = 0.8652(Chi square = 0.029); 95% CI: 30.82 - 36.82; df: 111). The value, 0.8652, is above 0.05 so we declare the result to be statistically non-significant. This signifies that, there is no correlation between the DFA results and gender in the studied population. Patients with the symptoms of meibomitis showed highest positivity (72.7%), whereas patients with dry eye showed least positivity (42.8%) for *C. trachomatis* antigen with DFA. Chlamydia elementary bodies appeared as round, bright, apple green, fluorescent particles, regular in outline (Figure 1). However, we did not observe any significant positivity difference in different age group/sex of the patients. Of the total 62 *C. trachomatis* positive cases 54(87.09%) had good clinical response for topical azithromycin (1% eye drops (twice a day) for 2 - 4 weeks) and topical lubricants and warm compresses.

Discussions

The underlying mechanism of MGD with follicular conjunctival inflammation is not yet clear; and the involvement of different bacteria in the pathophysiology of MGD is still a controversial subject. As per the conventional therapy of MGD is concerned, lid massage, lid expression and treatment with tetracycline and doxycycline, were used to treat MGD and relieving the symptoms [11,12]. Some of the recent studies had reported the effectiveness of topical azithromycin therapy for meibomian gland dysfunction and improving the signs and symptoms of the disease [11,13]. In a pilot study it was reported that, order of the neutral and polar lipid molecules gets altered in the disease state compared to normal state and treatment with azithromycin (antibacterial agent) can improve that abnormal condition towards normal by reducing the bacterial load in the eye lid [11,14]. Changes in the concentration of oleic acid in the meibum results in the increase in percentage of unsaturation of fatty acid associated with phospholipids in case of meibomitis was also reported in some studies [15,16]. In another pilot study over blepharitis, it was reported that, azithromycin provided significant improvement in signs and symptoms of blepharitis infections of the eyes [13,17].

Association of different bacteria with meibomian gland disorder have been reported earlier. It was

suggested that, MGD may be due to the effects of some of the bacteria themselves and their toxic metabolites [6]. Azithromycin is a broad-spectrum macrolide antibiotic against Gram positive, Gram negative, and atypical bacteria species, due its effective tissue penetration property, good pharmacokinetics for daily dose and a sustained delivery mechanism [18]. Currently the drug azithromycin is used widely in treatment of ocular and genital chlamydial infections and has been used in several trachoma control programs for mass antibiotic administration [17,18]. Azithromycin has also potent ocular anti-inflammatory properties but the mechanism behind its anti-inflammatory property is not clear [19]. In one of the pilot study it was reported that, azithromycin suppresses the activation of nuclear factor-kappa B and synthesis of pro-inflammatory cytokines in tracheal aspirate cells which make this antibiotic more effective than other antibiotics in treating the bacterial infections [20]. Besides this fact, azithromycin suppresses zymosan-induced production of pro inflammatory mediators by human corneal epithelial cells [21]. Currently trachoma prevalence in Indian population is ~6%, but in this group of meibomitis patients *C. trachomatis* was detected in 55% of patients signifying a possible role of *C. Trachomatis* in MGD with follicular conjunctival inflammation [22-24].

In sporadic follicular conjunctivitis patients *C. trachomatis* antigen detection rate varied between 22-28% [25]. Moreover the symptoms were relieved after azithromycin treatment in 87% of the patients thereby suggesting a possible association with *C. trachomatis* infections. Meibomian glands are the oil glands present in the margin of the eyelids. In a healthy individual secretion of meibomian gland consists of mainly neutral sterols and wax esters (nonpolar lipids), with lesser amounts of polar lipids, triglycerides, diesters, triesters, and free sterols [15]. Several changes had been observed in the meibomian lipid composition which result in MGD that includes, increased monounsaturated fatty acids (wax and sterol ester fatty acids) or other fatty acids [26,27]. Bacterial isolates found in MGD produce lipases that can alter the lipid composition which may in turn, enhance the growth of other microorganisms [26,8].

All these changes in the meibomian gland secretion results in abnormal meibum secretion from gland having higher melting temperature which results in thicker meibum, stagnation, ductal plugging, and pouting of the meibomian gland orifices resulting in meibomian gland dysfunction [28]. In previous studies topical azithromycin therapy relieved signs and symptoms of MGD and

restored the lipid properties of the abnormal meibomian gland secretion [11]. In this study since *C. trachomatis* was detected in more than half of the patients, it suggests a possible association.

Conclusion

We concluded from this pilot study that, *C. trachomatis* is supposed to be one of the major pathogenic bacteria for MGD with follicular conjunctival inflammation and 1% topical azithromycin (given as anti-chlamydial therapy) is a good choice for the treatment and improving the signs and symptoms of the disorder. As the role of chlamydia in MGD is not yet reported, this study will disclose an exciting fact about the pathophysiology of the disease, which will in turn improve treatment strategy to some extent to combat with the common but frustrating infection.

Ethical Approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institute.

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Conflicts of Interest

The authors declare that there are no conflicts of interest related to this work.

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