



ADULTS WITH DRY EYE DISEASE HAVE A HIGH RATE OF LATENT GRAM-NEGATIVE BACTERIA IN THEIR CONJUNCTIVA

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Abstract:

In youthful adults with parched eye disease, the prevalence of conjunctival *C. trachomatis* (CT), *M. hominis* (MH), and *U. urealyticum* (UU) contamination is investigated since these contamination may possible state contribute to long term subclinical illness. There were 57 subjects with DED (n = 57) and 49 subjects with non-dry eye control (n = 49) in the study. Self-recorded problem, bio microscopy, Schirmer I test, and division were used to diagnose the condition. The conjunctivas of all patients were scraped and analyzed with undeviating fluorescent to make a specific immune response assay equipment for the detection of CT, MH, and UU. DED patients were more likely to have at least one of the three microorganisms than controls, with 87.7% having at least one of the three. DED patients who had CT, MH, or UU infections accounted for 63.2%, 50.8%, and 42.1% of all cases. Infectious DED patients were found to have multiple pathogens in 65% of cases. Controls had a CT infection rate of 6.1%. A tall wave passing of *C. trachomatis*, *M. hominis*, and *U. urealyticum* was found in the conjunctiva of youthful adults with DED, suggesting that these infections could be significant risk factors for DED.

Keywords: Adults, Eye, Disease, Gram-Negative Bacteria

Introduction

Preposition the parched Eye Workshop (DEWS), parched eye disease involves inflammation along [1] with pathological processes, which is reflected in new therapeutic approaches being used to treat DED [2, 3]. The symptoms of chronic conjunctivitis and DED show similar long period of time indication of inflammation. These include conjunctival hyperemia, edema, and unimportant incusion. Unless special examination techniques are used, chronic conjunctivitis and DED are not significantly different in their diagnosis. There are links between DED and chronic conjunctivitis (especially allergic conjunctivitis), which may also be cause of DED [4]. DED may also be caused by chronic inflammation of the conjunctiva. It is thought that *C. trachomatis*, *M. hominis*, and *U. urealyticum* are the usual infectious bacterium that can persist for longer periods of time in the

human body and cause chronic low-grade nonspecific inflammation, which may be acute depending on the type [5-7].

As STD are predominantly found in youthful adults, these infectious agents tend to be found among them [8-10]. Furthermore, these pathogens, as well as closely related species, have been demonstrated to cause conjunctivitis when localized in the conjunctiva [11, 12]. As a consequence, this work sought to examine the regularity of conjunctival infections caused by *C. trachomatis*, *M. hominis*, *U. urealyticum* in young adults with DED.

Materials and methods

An entire of 106 subjects were comprised in the work, split into two groups, dry eye disease (57 subjects) and non-dry eye disease (49 subjects). These participants were aged 25 to 45, complained of dryness in their eyes, felt sand in the eyes, or felt foreign bodies in the eyes, had no significant conjunctival discharge or tears (either separately or in combination), had a Schirmer I test of 11 mm or less, and tear films that broke up within five seconds or smaller. Non-patched eye control subjects were in the same age group as the non-dry eye group. Severe conjunctivitis, pathological lacrimal passages, contact lens wear, refractive surgery in the past, and DED secondary to systemic diseases were excluded. In addition to endocrine disorder and systemic diseases of connective tissue, oral contraceptives, and current antibiotic, anti-inflammatory, cytostatic, and hormonal treatment regimens may be administered locally or systematically.

Ophthalmic Examination:

Conjunctival scrapings were collected for DFA and all patients underwent a comprehensive ophthalmic examination. In order to assess the severity of the disease, Schirmer's I and BUT tests were carryout on patients to determine the duration of the disease. Furthermore, these tests are widely available, highly sensitive, and highly specific. The Schirmer I test, for example, reaches 85% sensitivity and 100% specificity, while the BUT test reaches 83% and 85% [1]. These two tests were recommended by DEWS as part of a "practical sequence of tests" for detecting DED, on words with clinical history information, manifestation questionnaires, and ye surface staining categorized.

The lower conjunctival sac of the lateral eye was injected with a drop of topical proxymetacaine 0.5% before Schirmer's I test was carryout an analysis of the amount of wetting was performed five minutes later. After the instillation of sodium fluorescein dye, a slit lamp biomicroscope with cobalt blue filtered light was used to observe the tear film, and the interlude in middle of the last blink and the first break was recorded. Three repeated measurements were used to calculate individual average BUT values.

Sampling:

Using a topical proxymetacaine solution of 0.5% (Alcon-Couvreur), the conjunctival epithelial scraping was conducted in a standardized manner for each patient. The tarsal conjunctiva was examined four times before samples were collected. Following conjunctival scraping, methanol was applied to a slide and the material was fixed for seventy percent in the cold.

Direct Fluorescent Assay (DFA):

A specific trisaccharide component of cell wall lipopolysaccharide (LPS) is bound by antibodies to an epitope (aKdo-(2-8)-aKdo-(2-4)-aKdo) of *Ctrachomatis*, an exterior protein antigen for *M. hominis*, or a surface protein antigen for *U. urealyticum*; for the noticing of ocular *Ctrachomatis* contamination, only DFA tests have been void by the Food and Drug Administration [15]. Aside from its simplicity and cost-effectiveness, the DFA is also an excellent choice for routine use. Conjunctival scrapings are more sensitive than urogenital specimens, with 86-92% sensitivity and 96-999% specificity [16-18], respectively. Samples taken from the urogenital region can be 100% sensitive, while samples collected from the conjunctiva can be as specific as 96% or 99% [19]. As a result of the relative "purity" of conjunctival scrapings as compared to urogenital specimens, the

high sensitivity and specificity of the DFA in detecting ocular infection is attributed to this fact, and this is why the DFA obey more to discernment of infection in the previous than in the latter. This study chose the DFA method because of this reason. Based on the manufacturer's instructions, polyclonal antibody-hinge kits were used for detecting *C. trachomatis*, *M. hominis*, *U. urealyticum* antigens. A dark, humidified chamber and 30ml of Evans blue counterstain accommodate FITC conjugated antibodies was used to stain conjunctival scrape smears for 20 minutes at 20°C. A Leica DM2500 microscope (excitation wavelength: 490 nm; emission wavelength: 520 nm) equipped with FITC fluorescence was used to examine specimens after they had been washed with PBS and twice in distilled water, dried, and covered with 10% glycerin solution in PBS. In *C. trachomatis* diagnostic tests, the domination was a heteroploidy line of L929 mouse fibroblasts infected with *C. trachomatis* strain L2. A scrape sample was evaluated if it contained at least 50 epithelial cells. At a magnification of 400x, specific fluorescence spots were visible. The manufacturer describes this pattern as specific and some authors have discussed it [20, 21]. Several studies have found this criterion to provide a good balance between sensitivity and specificity, so at least ten loci of specific fluorescence were required to qualify a sample as positive [16–18]. Patients were considered infected if they had a unocular infection.

Statistical analysis:

Statistica for Windows 6.0 was used to analyze data in a nonparametric manner. The demographic parameters and results of the groups were compared using the Mann-Whitney U test and Fisher's exact test. P 0.05 was considered statistically significant.

Table 1: Patients in the dry eye disease group and the control group are characterized by the following characteristics

	DED (n = 57)	Non-dry eye controls (n = 49)	P - Value
Age in years, mean $\pm$ SD	34.7 $\pm$ 6.4	34.5 $\pm$ 6.9	0.52
Sex, male/female	24/33	23/26	0.07
Schirmer's I test, mm	7.7 $\pm$ 0.9	14.9 $\pm$ 1.2	<0.01
BUT, seconds	3.6 $\pm$ 0.7	10.9 $\pm$ 0.9	<0.01

Results

Ophthalmic Examination Results and Patient Characteristics. DED and control groups showed no statistically significant differences in demographic characteristics (Table 1). According to Schirmer's I and BUT tests, all patients with DED had lessened tear up making and a destabilized tear up film compatible with the indications. There were no differences in these characteristics between the control group and the experimental group. No complaints or symptoms of DED were present in these patients. Ninety.2% of the patients in the DED group reported a mean time of the disease of 41.16 $\pm$ 9.12 months (range 37-58 months). There was a slow increase in the level of symptoms reported over time during the period when 9.8% of the patients were studied; the symptoms reported by 9.8% of the subjects ranged from 12 to 26.4 months.

DFA Results.

As compared with the control group, 50 of the DED patients had one or more of the microorganisms examined, versus 4 (8.1%). From all DED cases, only 35% were diagnosed with a single agent. Study participants who were also infected with *C. trachomatis* were 86.2% (72% for all infected study participants and 63.2% for all infected study participants). The non-dry eye control group also had 4 (8.2 %) cases of *C. trachomatis*, either alone or in consortium with further species. Infected controls did not show symptoms of long-term conjunctivitis or dry eye during ophthalmic examination.

Discussion

Researchers found that a significant portion of people aged 25 to 45 with decreased tear production, destabilized tear film, hyperemia of the conjunctiva, and remonstrance usual of DED have chronic infectious conjunctivitis origin by infection with *C. trachomatis*, *M. hominis*, or *U. urealyticum*, alone or in combination. Domont conjunctival infection is another important risk factor for dry-eye patients of this age group, according to the statement that dry-eye patients have mild conjunctivitis [22]. A risk factor that has not been identified can only be responsible when DED occurs in youthful adults wanting any obvious possibility factors. Because the clinical picture and complaints do not match those associated with infectious damage to the conjunctiva, this chronic conjunctival infection is diagnosed as DED and not as conjunctivitis. In addition to contributing to the incidence of DED, such latent conjunctival infections may require specific diagnostic and treatment strategies. There was a complete masking of clinical symptoms of chronic conjunctival inflammation caused by *C. trachomatis*, *M. hominis*, and *U. urealyticum* in this study compared to symptoms of acute conjunctivitis (acute conjunctivitis was excluded from enrollment). DED is not a universally exhibited symptom in all patients found to be infected (notably, infected controls did not present with the symptoms). A number of factors are likely to contribute to conjunctival inflammation, including (1) early stage of the disease and (2) genetic factors [23, 24]. It is also possible that the pathogen has genetic variability, which is mostly the case with *C. trachomatis* [23]. A limitation of the study is that serotyping was not performed. *C. trachomatis* species or individual serovars within it are not known to be associated with DED cases [23]. Further, no investigation was undertaken into other conjunctival bacterial microflora that may play some role in DED risk. DED is unlikely to be associated with microorganisms which cause no inflammation in normal (saprophytic) conjunctival microflora. Conjunctivitis caused by infectious agents being pathogenic for the conjunctiva, on the other hand, normally would have a characteristic clinical picture, but this was not observed in DED patients. The control of the conjunctival bacterial microflora in these patients was not performed because these microorganism species were unlikely to contribute to DED risk in these patients. Nevertheless, nonpathogenic and opportunistic microorganisms are more likely to colonize the conjunctiva during inflammation associated with DED. *C. trachomatis*, for example, is very contagious and obligate pathogenic. These findings tend to indicate that secondary colonization of already inflamed conjunctiva is not likely to occur, but that these pathogens may play a primary role in causing and maintaining inflammation; more research is needed on these matters, however. There is already evidence of an association between DED and certain infectious agents, such as HIV, HCV, hepatitis C virus, and Epstein-Barr virus. Chronic viral infections initiate or contribute to lacrimal gland dysfunction in Sjogren's syndrome via autoimmune reactions [25]. Alves et al. [25] reviewed those studies and discussed autoimmune mechanisms rather than direct damage to conjunctiva and lacrimal glands. Even so, chronic infection of the conjunctiva and non-Sjogren's dry eye remain poorly understood, with the latter causing the majority of dry eye prevalence [26]. Recently, the connection between DED and Chlamy-dophila pneumoniae infection in simultaneous clinical signs of follicular conjunctivitis has been reported, and it has been demonstrated that the agent is localized on the conjunctiva and partial efficacy of etiotropic therapy was achieved [27]. We did not examine the association between unilaterally detected infection and manifestations of DED, because localization of the infectious agent in only one eye is considered unlikely. There are several reasons for this unlikelihood, one of which is that DFA interpretation is specific, which requires detection of at least a certain number of loci exhibiting specific fluorescence, thereby resulting in false-positive results (e.g., those for the contralateral eye). There may be differences in the prevalence of a particular infectious agent according to the population. Infections caused by *C. trachomatis* may be more prevalent in individuals aged 25–45 due to an increased risk of urogenital infections [8–10]. In accordance with the International Dry Eye Work Shop, evaporative dry eye can be classified into two subcategories: (1) aqueous-deficient dry eye, and (2) aqueous-deficient dry eye; nevertheless, the etiopatho-genetic subcategory described in this study is a combination of both types [1]. Ocular surface disease and lacrimal deficiency make up the subcategory of DEWS.

Conclusion

It has been shown that latent *C.trachomatis*, *M.hominis*, *U.urealyticum* infections are detected in the conjunctiva of young adults with DED and that this may be an important risk factor for this disease. In addition to the potentially damaging effects they can have on the conjunctiva, they are also more prevalent among this age group. As a result, it is deemed appropriate to conduct an examination for latent infections and, possibly, further antimicrobial treatment for some patients with DED.

References

1. International Dry Eye Workshop. The definition and classification of dry eye disease: Report of the definition and classification subcommittee of the International Dry Eye Workshop (2007). *The Ocular Surface* 2007, 5(2), 75–92. [https://doi.org/10.1016/S1542-0124\(12\)70081-4](https://doi.org/10.1016/S1542-0124(12)70081-4)
2. Rao, S. N. Topical cyclosporine 0.05% for the prevention of dry eye disease progression. *J. Ocular Pharmacol. Ther.* 2010, 26(2), 157–164. <https://doi.org/10.1089/jop.2009.0119>
3. Amparo, F.; Dastjerdi, M. H.; Okanobo, A.; et al. Topical interleukin 1 receptor antagonist for treatment of dry eye disease: A randomized clinical trial. *J. Am. Med. Assoc. Ophthalmol.* 2013, 131(6), 715–723. <https://doi.org/10.1001/jamaophthalmol.2013.1929>
4. Hom, M. M.; Nguyen, A. L.; Bielory, L. Allergic conjunctivitis and dry eye syndrome. *Ann. Allergy Asthma Immunol.* 2012, 108(3), 163–166. <https://doi.org/10.1016/j.anai.2011.12.006>
5. Beatty, W. L.; Byrne, G. I.; Morrison, R. P. Repeated and persistent infection with Chlamydia and the development of chronic inflammation and disease. *Trends Microbiol.* 1994, 2(3), 94–98. [https://doi.org/10.1016/0966-842X\(94\)90079-4](https://doi.org/10.1016/0966-842X(94)90079-4)
6. Razin, S.; Yogev, D.; Naot, Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol. Biol. Rev.* 1998, 62(4), 1094–1156. <https://doi.org/10.1128/MMBR.62.4.1094-1156.1998>
7. Baka, S.; Kouskouni, E.; Antonopoulou, S.; et al. Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* in women with chronic urinary symptoms. *Urology* 2009, 74(1), 62–66. <https://doi.org/10.1016/j.urology.2009.02.051>
8. McCormack, W. M. Epidemiology of *Mycoplasma hominis*. *Sex. Transm. Dis.* 1983, 10(4, Supplement), 261–262. <https://doi.org/10.1097/00007435-198310000-00002>
9. Goller, J. L.; Ward, J.; Saunders, M.; et al. Chlamydia sentinel surveillance in Aboriginal community controlled health services finds higher testing and positivity rates among younger people. *Aust. N. Z. J. Public Health* 2012, 36(6), 577–581. <https://doi.org/10.1111/j.1753-6405.2012.00955.x>
10. Salmeri, M.; Valenti, D.; la Vignera, S.; et al. Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* infection in unselected infertile men. *J. Chemother.* 2012, 24(2), 81–86. <https://doi.org/10.1179/1973947812Y.0000000031>
11. Malamos, P.; Georgalas, I.; Rallis, K.; et al. Evaluation of single-dose azithromycin versus standard azithromycin/doxycycline treatment and clinical assessment of regression course in patients with adult inclusion conjunctivitis. *Curr. Eye Res.* 2013, 38(12), 1198–1206. <https://doi.org/10.3109/02713683.2013.833874>
12. Bjõrnelius, E.; Jensen, J. S.; Lidbrink, P. Conjunctivitis associated with *Mycoplasma genitalium* infection. *Clin. Infect. Dis.* 2004, 39(7), e67–e69. <https://doi.org/10.1086/423848>
13. American Association for Research in Vision and Ophthalmology. *Helsinki Declaration*. https://www.arvo.org/About_ARVO/Policies/Helsinki_Declaration/.
14. Hindawi. *ISRN Ophthalmology Guidelines*. <https://www.hindawi.com/journals/isrn.ophthalmology/guidelines/>.
15. By, P.; Papp, J. R.; Schachter, J.; Gaydos, C. A.; van der Pol, B. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*—2014. *Morb. Mortal. Wkly. Rep. Recommendations Rep.* 2014, 63(2), 1–19. <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6302a1.htm>
16. Sachdeva, P.; Patel, A. L.; Sachdev, D.; Ali, M.; Mittal, A.; Saluja, D. Comparison of an in-house PCR assay, direct fluorescence assay and the Roche AMPLICOR *Chlamydia trachomatis*

- kit for detection of *C. trachomatis*. *J. Med. Microbiol.* 2009, 58(7), 867–873. <https://doi.org/10.1099/jmm.0.008342-0>
17. Stamm, W. E.; Harrison, H. R.; Alexander, E. R.; Cles, L. D.; Spence, M. R.; Quinn, T. C. Diagnosis of *Chlamydia trachomatis* infections by direct immunofluorescence staining of genital secretions: A multicenter trial. *Ann. Intern. Med.* 1984, 101(5), 638–641. <https://doi.org/10.7326/0003-4819-101-5-638>
18. Kiviat, N. B.; Wolner-Hanssen, P.; Peterson, M.; et al. Localization of *Chlamydia trachomatis* infection by direct immunofluorescence and culture in pelvic inflammatory disease. *Am. J. Obstet. Gynecol.* 1986, 154(4), 865–873. [https://doi.org/10.1016/0002-9378\(86\)90212-7](https://doi.org/10.1016/0002-9378(86)90212-7)
19. Potts, M. J.; Paul, I. D.; Roome, A. P.; Caul, E. O. Rapid diagnosis of *Chlamydia trachomatis* infection in patients attending an ophthalmic casualty department. *Br. J. Ophthalmol.* 1986, 70(9), 677–680. <https://doi.org/10.1136/bjo.70.9.677>
20. Zigangirova, N. A.; Rummyantseva, Y. P.; Morgunova, E. Y.; et al. Detection of *C. trachomatis* in the serum of patients with urogenital chlamydiosis. *BioMed. Res. Int.* 2013, Article ID 489489, 7 pages. <https://doi.org/10.1155/2013/489489>
21. Patton, D. L.; Chan, K. Y.; Kuo, C.-C.; Cosgrove, Y. T.; Langley, L. In vitro growth of *Chlamydia trachomatis* in conjunctival and corneal epithelium. *Invest. Ophthalmol. Vis. Sci.* 1988, 29(7), 1087–1095. <https://doi.org/10.1167/iovs.29.7.1087>
22. Uchida, H.; Imanaga, Y. Effect of mild conjunctivitis complication on tear balance in dry eye. *Contact Lens Anterior Eye* 2012, 35(5), 240–242. <https://doi.org/10.1016/j.clae.2012.06.002>
23. Abdelsamed, H.; Peters, J.; Byrne, G. I. Genetic variation in *Chlamydia trachomatis* and their hosts: Impact on disease severity and tissue tropism. *Future Microbiol.* 2013, 8(9), 1129–1146. <https://doi.org/10.2217/fmb.13.102>
24. Hu, V. H.; Holland, M. J.; Burton, M. J. Trachoma: Protective and pathogenic ocular immune responses to *Chlamydia trachomatis*. *PLoS Negl. Trop. Dis.* 2013, 7(2), Article ID e2020. <https://doi.org/10.1371/journal.pntd.0002020>
25. Alves, M.; Angerami, R. N.; Rocha, E. M. Dry eye disease caused by viral infection: Review. *Arq. Bras. Oftalmol.* 2013, 76(2), 129–132. <https://doi.org/10.5935/0004-2749.20130033>
26. Akpek, E. K.; Klimava, A.; Thorne, J. E.; Martin, D.; Lekhanont, K.; Ostrovsky, A. Evaluation of patients with dry eye for presence of underlying Sjögren syndrome. *Cornea* 2009, 28(5), 493–497. <https://doi.org/10.1097/ICO.0b013e31818d62a4>
27. Krásný, J.; Hrubá, D.; Netuková, M.; Kodat, V.; Pokorná, J. Keratoconjunctivitis sicca during follicular conjunctivitis in adult patients with *Chlamydia pneumoniae* etiology (summarizing twelve years study). *Ceska Slovenska Oftalmologie* 2011, 67(2), 42, 44–48, 50. [Czech].