Association of Conjunctival Bacterial Infection and Female Sex in Cicatricial Trachoma

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Purpose. Conjunctival infection with non-chlamydial bacteria may play an important role in the progression of trachoma, especially with regard to the development of corneal opacity and blindness. To further characterize the microbiological profile of bacterial conjunctival infections in cicatricial trachoma, a conjunctival swabbing of adults in rural Ethiopia was performed.

Methods. In a cross-sectional study conducted in nine Ethiopian villages with hyperendemic trachoma, persons 40 years of age or older with signs or symptoms consistent with trichiasis were recruited and conjunctival swabbing for bacterial pathogens was performed.

Results. Conjunctival examination and swabbing on 112 females and 36 males were performed. Of the 148 study participants, 101 (68.2%) were confirmed to have trichiasis, and 118 (80%) had conjunctival swabs positive for bacteria. In multivariate analyses, growth of pathogenic conjunctival bacteria was independently associated with trichiasis (odds ratio [OR] 6.93; 95% confidence interval [CI] 2.71–17.7) and female sex (OR 5.90; 95% CI 2.09–16.7). Females were more likely to have swabs positive for *Streptococcus pneumoniae* or *Haemophilus influenzae* than were males (OR 9.09; 95% CI 1.17–70.8).

Conclusions. In a region of Ethiopia with endemic trachoma, conjunctival bacterial growth was more common in females than that in males. *S. pneumoniae* and *H. influenzae*, both of which frequently colonize the nasopharynx of children, were more common in females, suggesting that the preponderance of infection in females may be attributable to close contact with children. This finding is consistent with the theory that childcare activities may preferentially expose females to ocular chlamydial infection. (ClinicalTrials.gov number, NCT00221364.) (*Invest Ophthalmol Vis Sci.* 2012;53:5208–5212) DOI:10.1167/iovs.12-9984

Trachoma is the leading infectious cause of blindness worldwide.[1] Ocular chlamydial infection results in conjunctival inflammation, and repeated episodes of infection lead to conjunctival scarring, entropion, and trichiasis, which in turn increases the risk for corneal opacities and blindness.[2] These progressive cicatricial changes occur many years after ocular chlamydial infection, for reasons that are not entirely clear. Even in places where ocular chlamydial infection has been reduced, the risk of trichiasis and blindness persists. According to recent estimates, trachomatous trichiasis affects 8.2 million people worldwide.[3]

It is known that the conjunctivae of eyes with trachomatous trichiasis are frequently colonized with bacteria.[4–6] Case-control studies have shown that conjunctival bacterial infection is associated with trichiasis and conjunctival scarring, although the significance of this bacterial growth is unclear.[7,8] Eyelashes touching the globe (i.e., trichiasis) could provide a direct route for environmental pathogens to gain entry to the conjunctiva or cornea. Once these organisms colonize the conjunctiva, they may increase the risk for corneal infections, which could explain the increased incidence of corneal opacities in patients with trichiasis.[6,9] In addition, some have suggested that non-chlamydial bacterial infections of the conjunctiva could play a role in progressive conjunctival cicatrization, perhaps explaining why progression of scarring can occur in the absence of ocular chlamydial infection.[4,7,8]

A better understanding of the conjunctival flora of patients with trachoma may provide clues about the importance of non-chlamydial bacterial infections for this disease. We performed a clinical trial of different mass azithromycin treatment strategies in an area of Ethiopia with highly prevalent trachoma.[10,11] During this study, we collected swabs of persons with cicatricial signs of trachoma, to determine the microbiologic profile of conjunctival organisms in persons with trichiasis.

Methods

In March 2004, we performed a cross-sectional study in nine villages from two *woredas* (districts) of the Gurage Zone, Ethiopia (Enemor and Ener woreda, and Goro woreda). The study area was hyperendemic for trachoma, with estimates of prevalent ocular chlamydial infection as high as 63.5% among 1- to 5-year-old children.[12] Villages were randomly selected from a sample of 24 villages that were being monitored as part of a clinical trial for trachoma (ClinicalTrials.gov number NCT00221364). Villages came from different treatment arms of the trial, but were similar in that none of the villages had received

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mass azithromycin within the preceding 6 months. Of the nine villages, four had received a single dose of mass azithromycin 1 year prior,[10] three had received mass azithromycin 1 year prior and 6 months prior to this study,[11] and two had received no mass azithromycin treatment.

We employed a community leader from each village to recruit all persons 40 years of age or older with signs or symptoms of trichiasis. Trichiasis is relatively common in these communities, and all community leaders were familiar with this condition. Before the recruitment started, ophthalmic nurses reviewed the signs and symptoms of trichiasis with community leaders, and demonstrated the physical findings with photographs. We focused on persons 40 years of age or older, since the vast majority of trichiasis patients fall within this age group.[13] Recruited study participants who consented to be in the study presented to a central location for conjunctival examination and swabbing. Trained staff assessed the right eye of each individual for the clinical signs of trachoma using ×2.5 binocular loupes, according to the WHO simplified grading system.[14] Of particular interest were the grades for cicatricial trachoma: trachomatous scarring (TS), defined as the presence of easily visible scarring in the everted upper tarsal conjunctiva, and trachomatous trichiasis (TT), defined as the presence of at least one eyelash touching the globe. Individuals with trichiasis were referred to the ORBIS trichiasis surgery program. After examination, we swabbed the unanesthetized right lower conjunctival fornix of each study participant with a sterile polyester-tipped applicator, and placed the swab in skim milk–tryptone–glucose–glycerine (STGG) media.[15] We collected one negative field control swab from a randomly selected study participant in each village by passing the swab 1 inch in front of, but not touching, the conjunctiva. Swabs were stored on ice in the field, then at −20°C until transported at −4°C to San Francisco, where they were stored at −70°C for up to 6 months until processed. We placed 100 μL of thawed and vortexed STGG media on blood and chocolate agar plates, then streaked for isolation, and incubated the plates for at least 48 hours at 35°C with 5% CO_2. Bacterial isolates were identified according to standard microbiological techniques. The quantity of each isolate was assessed using a 4-point scale as follows: rare (1), few (2), moderate (3), or many (4). The microbiologist was masked to all clinical information for each swab.

We classified viridans group *Streptococci*, coagulase-negative *Staphylococcus* spp., *Corynebacterium* spp., and *Bacillus* spp. as commensal conjunctival flora, and all other bacterial species as pathogenic bacterial species. We classified *Streptococcus pneumoniae* and *Haemophilus influenzae* as common nasopharyngeal species, and *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. as common enteric species. We displayed the proportion of study participants with each bacterial isolate, stratified by trichiasis status and sex. Predictors of the presence of conjunctival bacteria were assessed using mixed-effects logistic regression with village as a random effect; univariate and multivariate analyses were constructed using age, female sex, and trichiasis as predictors. We calculated the average quantity of bacteria for each study participant based on the 4-point scale outlined above and assessed whether this score was different in females versus males, and in persons with and without trichiasis, using mixed-effects linear regression with village as a random effect. All analyses were performed with commercial data analysis and statistical software (Stata 10; StataCorp LP, College Station, TX).

The study was approved by the Committee for Human Research at the University of California, San Francisco and the Ethiopian Science and Technology Commission, and adhered to the tenets of the Declaration of Helsinki.

Results

We examined and swabbed 113 females and 36 males 40 years of age or older from the nine study villages. Of the 149 swabbed persons, 148 had trachomatous scarring and are included in this report. The excluded individual was a 50-year-old female who grew *H. influenzae* and viridans group *Streptococcus* spp. Of the 148 study participants, 101 were confirmed to have trichiasis (Table 1). We identified 257 different conjunctival bacterial isolates from 118 (79.7%) study participants (Table 2). Of the total isolates, 160 (62.3%) were classified as normal commensal organisms, defined as group viridans Streptococcus spp., coagulase-negative *Staphylococcus* spp., *Corynebacterium* spp., or *Bacillus* spp., and the remainder were classified as pathogenic. Each bacterial isolate was judged for quantity of organisms based on a 4-point scale; the average quantity score per study participant was 1.74 (95% confidence interval [CI] 1.44–2.02). Of nine negative field control swabs, a single swab was positive, growing few *Corynebacterium* spp.

Conjunctival bacterial infection did not vary significantly by age, but was more common in females and in persons with trichiasis (Table 3). Females were more likely than males to harbor commensal and pathogenic conjunctival bacteria, as well as the organisms most commonly found in the nasopharynx of children (*S. pneumoniae* and *H. influenzae*). Similarly persons with trichiasis were more likely to have cultures positive for pathogenic organisms, nasopharyngeal organisms, and commensal organisms. After adjusting for trichiasis, the burden of bacteria was higher for females compared with males (average quantity score 1.79 [95% CI 1.57–2.01] vs. 1.41 [95% CI 1.08–1.74]; $P = 0.08$). The quantity of bacteria in trichiatic eyes was higher compared with those without trichiasis (sex-adjusted average quantity score 1.86 [95% CI 1.64–2.08] vs. 1.35 [95% CI 1.03–1.67]; $P = 0.003$).

In multivariate mixed-effects logistic regression models, trichiasis and sex were significant independent predictors of conjunctival bacterial growth (Table 3). Female sex was strongly associated with the presence of bacteria normally found in the nasopharynx of children (odds ratio [OR] 9.09; 95% CI 1.17–70.8). In contrast, female sex was not associated with the presence of commensal conjunctival flora (OR 1.57; 95% CI 0.58–4.25). Treatment arm was not a significant

Table 1. Characteristics of Study Participants, Gurage Zone, Ethiopia, 2004

Characteristic	Males, No. (%) ($n = 36$)	Females, No. (%) ($n = 112$)
Age, y		
40–49	15 (41.7)	51 (45.5)
50–59	9 (25.0)	27 (24.1)
≥60	12 (33.3)	34 (29.8)
Residence		
Village 1	5 (13.9)	15 (13.4)
Village 2	0 (0)	3 (2.7)
Village 3	0 (0)	20 (17.9)
Village 4	0 (0)	5 (4.5)
Village 5	7 (19.4)	17 (15.2)
Village 6	14 (38.9)	24 (21.4)
Village 7	9 (25.0)	8 (7.1)
Village 8	1 (2.8)	14 (12.5)
Village 9	0 (0)	6 (5.4)
Trachoma grade*		
TF	0 (0)	0 (0)
TI	0 (0)	0 (0)
TS, no TT	18 (50.0)	29 (25.9)
TS and TT	18 (50.0)	83 (74.1)

* According to the WHO simplified grading system. TF, follicular trachomatous inflammation; TI, intense trachomatous inflammation; TS, trachomatous scarring; TT, trachomatous trichiasis.[14]

Table 2. Conjunctival Flora of Study Participants Stratified by Trichiasis Status and Sex, Gurage Zone, Ethiopia, 2004

Organism	Trichiasis Present				Trichiasis Absent			
	Male ($n = 18$)		Female ($n = 83$)		Male ($n = 18$)		Female ($n = 29$)	
Individual organisms								
Commensal								
Group viridans *Streptococcus* spp.	11	(61.1%)	52	(62.7%)	3	(16.7%)	13	(44.8%)
Corynebacterium spp.	5	(27.8%)	35	(42.2%)	4	(22.2%)	4	(13.8%)
Coagulase-negative *Staphylococcus* spp.	8	(44.4%)	15	(18.1%)	3	(16.7%)	1	(3.4%)
Bacillus spp.	1	(5.6%)	4	(4.8%)	0	(0%)	1	(3.4%)
Pathogenic gram positive								
Streptococcus pneumonia	1	(5.6%)	15	(18.1%)	0	(0%)	2	(6.9%)
Staphylococcus aureus	0	(0%)	6	(7.2%)	2	(11.1%)	1	(3.4%)
Catalase-negative gram-positive cocci	0	(0%)	5	(6.0%)	1	(5.6%)	0	(0%)
Group A *Streptococcus* spp.	0	(0%)	4	(4.8%)	0	(0%)	0	(0%)
Group F *Streptococcus* spp.	0	(0%)	2	(2.4%)	0	(0%)	0	(0%)
Abiotrophia sp.	0	(0%)	1	(1.2%)	0	(0%)	0	(0%)
Nonhemolytic *Streptococcus* spp.	0	(0%)	1	(1.2%)	0	(0%)	0	(0%)
Gram-positive rods	0	(0%)	0	(0%)	1	(5.6%)	0	(0%)
Pathogenic gram negative								
Nonenteric gram-negative rods	1	(5.6%)	18	(21.7%)	1	(5.6%)	2	(6.9%)
Haemophilus influenzae	0	(0%)	19	(22.9%)	0	(0%)	0	(0%)
Escherichia coli	0	(0%)	3	(3.6%)	0	(0%)	1	(3.4%)
Moraxella (*Branhamella*) *catarrhalis*	0	(0%)	2	(2.4%)	1	(5.6%)	1	(3.4%)
Klebsiella pneumonia	1	(5.6%)	2	(2.4%)	0	(0%)	0	(0%)
Haemophilus sp., noninfluenzae	0	(0%)	1	(1.2%)	0	(0%)	0	(0%)
Moraxella sp.	0	(0%)	1	(1.2%)	0	(0%)	0	(0%)
Enterobacter	0	(0%)	1	(1.2%)	0	(0%)	0	(0%)
Organism classification								
Any organism	14	(77.8%)	78	(94.0%)	9	(50.0%)	17	(58.6%)
All commensal organisms*	11	(61.1%)	19	(22.9%)	6	(33.3%)	12	(41.4%)
Any commensal organism*	14	(77.8%)	70	(84.3%)	7	(38.9%)	13	(44.8%)
Any pathogenic organism*	3	(16.7%)	59	(71.1%)	3	(16.7%)	5	(17.2%)
1 Pathogenic organism	3	(16.7%)	40	(48.2%)	1	(5.6%)	4	(13.8%)
2 Pathogenic organisms	0	(0%)	14	(16.9%)	1	(5.6%)	0	(0%)
≥3 Pathogenic organisms	0	(0%)	5	(6.0%)	1	(5.6%)	1	(3.4%)
Nasopharyngeal organism†	1	(5.6%)	26	(31.3%)	0	(0%)	2	(6.9%)
Enteric organism‡	1	(5.6%)	6	(7.2%)	0	(0%)	1	(3.4%)

Data refer to the number and proportion of study participants. Some study participants had more than one bacteria isolated, so numbers do not sum to 100%.

* Commensal organisms defined as group viridans *Streptococcus* spp., coagulase-negative *Staphylococcus* spp., *Corynebacterium* spp., or *Bacillus* spp.; all others considered pathogenic.

† *S. pneumoniae* or *H. influenza*.

‡ *E. coli*, *K. pneumoniae*, or *Enterobacter*.

predictor for any of the conjunctival bacteria outcomes, and was therefore not included in the final models.

Discussion

We confirmed here that persons with trachomatous trichiasis frequently have conjunctival bacterial infection, often with multiple pathogenic species. Persons with trichiasis were more likely to have conjunctival bacterial infection than those without trichiasis, and females were more likely to be infected than males. Females were especially likely to be infected with *S. pneumoniae* and *H. influenzae*, which are organisms commonly found in the nasopharynx of young children.

We detected at least one species of bacteria in 80% of persons 40 years of age and older in this study. This overall yield is consistent with several recent studies from industrialized countries that collected conjunctival swabs from normal eyes before ophthalmic procedures, although the proportion of isolates classified as pathogenic was higher in this Ethiopian population (38% of isolates) compared with these other studies (<20% of isolates).[16–18] The higher proportion of pathogenic bacteria could result from poverty and lack of medical services in the study population. In support of this theory, a study of normal eyes from a rural population in a different developing country, Sierra Leone, found that the majority of conjunctival bacterial isolates would be classified as pathogenic.[19] Alternatively, pathogenic bacteria may be more likely to colonize the conjunctiva of eyes with ocular surface disease. Indeed, the eyes in this study were not normal: virtually all had trachomatous scarring, and two thirds had trichiasis. These

Table 3. Trichiasis and Female Sex as Predictors of Conjunctival Bacterial Growth among Persons 40 Years of Age and Older in Nine Villages in Gurage Zone, Ethiopia, 2004

	Any Commensal Organism*		Any Pathogenic Organism*		Nasopharyngeal Organism†	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Univariate models						
Female sex	2.58 (1.06–6.31)	0.04	7.58 (2.72–21.2)	<0.001	11.7 (1.53–89.1)	0.02
Trichiasis	6.66 (3.03–14.6)	<0.001	8.27 (3.27–20.9)	<0.001	8.21 (1.86–36.2)	0.005
Age, per decade	0.99 (0.71–1.40)	0.97	1.10 (0.81–1.51)	0.54	1.10 (0.77–1.56)	0.62
Multivariate model						
Female sex	1.57 (0.58–4.25)	0.38	5.90 (2.09–16.7)	0.001	9.09 (1.17–70.8)	0.04
Trichiasis	6.15 (2.70–14.0)	<0.001	6.93 (2.71–17.7)	<0.001	6.59 (1.47–29.6)	0.01
Age, per decade	1.07 (0.75–1.53)	0.71	1.22 (0.86–1.73)	0.27	1.09 (0.75–1.59)	0.65

Mixed-effects logistic regression, with village as a random effect.

* Commensal organisms defined as group viridans *Streptococcus* spp., coagulase-negative *Staphylococcus* spp., *Corynebacterium* spp., or *Bacillus* spp.; all others considered pathogenic.

† *S. pneumoniae* or *H. influenza*.

ocular abnormalities may make conjunctival colonization with pathogenic bacteria more likely. Trichiatic lashes could provide a direct route for conjunctival inoculation. Consistent with this theory, conjunctival infections in other cicatrizing conjunctival disorders, such as mucous membrane pemphigoid and Stevens–Johnson Syndrome, are predominantly caused by pathogenic organisms.[20]

In this study, females were much more likely than males to have conjunctival bacterial infection. Even after adjusting for age and the presence of trichiasis, females were almost 6-fold more likely to be infected with a pathogenic species of bacteria, and roughly 9-fold more likely to be infected with the organisms most commonly found in the nasopharynx of children: *S. pneumoniae* and *H. influenzae*. These results suggest that females are at increased risk for bacterial conjunctival infection because they are the primary caregivers for children. Studies in several African countries have shown that the proportion of children with nasopharyngeal carriage of *S. pneumoniae* and *H. influenzae* far exceeds that of adults, with pneumococcal carriage exceeding 90% in some studies.[21–24] Because of their childcare role, females have frequent direct exposure to these nasopharyngeal organisms, which likely increases their risk of nasopharyngeal and conjunctival infection.[25,26] Organisms not associated with childcare, such as commensal conjunctival pathogens and enteric pathogens, were not significantly more common among females, suggesting that childcare in particular may increase the risk of conjunctival infection for females.

Numerous studies in the trachoma literature have demonstrated that females living in trachoma-endemic areas have more conjunctival inflammation, scarring, and trichiasis than do males, presumably because of conjunctival infection with chlamydia.[27–29] However, studies that have assessed for ocular chlamydial infection in adults have not found more infection in females compared with males.[30,31] It is unclear why this is the case, but because the duration of ocular chlamydial infection decreases with age, a cross-sectional study may not detect much infection among adults.[32] Our study, although not providing evidence of more chlamydial infection among females, does support the plausibility of this theory. Just as *S. pneumoniae* and *H. influenzae* are more common among children than among adults, so too is ocular *C. trachomatis*.[30,31,33,34] Females are likely at increased risk for acquiring both nasopharyngeal organisms and ocular chlamydia during the course of their childcare duties, which may explain the increased prevalence of conjunctival scarring among adult females in areas with endemic trachoma.[35,36]

Some have suggested that non-chlamydial bacterial infections may form another causative pathway for trachomatous scarring and trichiasis.[7,8] Although this cross-sectional study cannot comment on the directionality of the association between bacterial infection, trichiasis, and sex, we note that our findings could also be explained by this theory.

This study has several limitations. Our findings may have limited generalizability, since we conducted this study in an area with highly prevalent trachoma and targeted those persons in the community who had signs of severe disease. Moreover, this was not a population-based study; bias could have been introduced if the individuals who chose to participate in the study were different from those who chose not to participate. Bacterial growth on one of the negative control swabs leaves open the possibility that some of the bacterial isolates were contaminants from the field or the laboratory. However, we do not think this is likely, since this negative control swab grew only a small amount of a commensal organism. We did not perform swabbing for ocular chlamydia, so we could not examine the relationship between conjunctival chlamydial infection and conjunctival infection with other bacteria. However, in similar settings the prevalence of ocular chlamydia in older individuals has been quite low, so swabbing for chlamydia may not have provided much additional information.[10,37] Finally, the cross-sectional study design can offer no insight into causality, especially regarding whether conjunctival bacterial infections increase the risk of cicatricial trachomatous changes or whether scarred conjunctivae are simply more likely to harbor bacteria.

In conclusion, we showed that in a population of adults with cicatricial trachoma, bacterial conjunctival infections were common, and that a substantial proportion of individuals were infected with pathogenic organisms. Infection was more common in persons with trichiasis compared with those without. Infection was also much more common among females than among males, likely because of increased exposure to infected children during childcare. These findings are consistent with the theory that childcare preferentially exposes females to ocular chlamydia.

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