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Bacterial Infection and Trachoma in The Gambia: A Case–Control Study

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PURPOSE. Trachoma is the leading infectious cause of blindness worldwide. Conjunctival scarring is initiated by recurrent *Chlamydia trachomatis* infection. However, disease progression to trichiasis occurs even in regions where chlamydial prevalence is currently low, which suggests that other factors, for example other bacterial infection, may also drive inflammation and scarring, particularly in the late stages of trachoma. This study was undertaken to investigate whether trachomatous trichiasis or conjunctival scarring are associated with increased prevalence of bacterial infection.

METHODS. Within a case–control study design, individuals with trichiasis or conjunctival scarring (without trichiasis) were compared with normal matched control subjects. Subjects were examined for signs of trachoma. Conjunctival swab samples were collected for bacteriologic culture and *C. trachomatis* PCR.

RESULTS. Recruited for the study were 121 trichiasis case–control pairs and 117 conjunctival scarring case–control pairs. Eyes with trichiasis were more frequently infected with bacteria (37%) than were normal control eyes (7%) (OR: 8.2; $P < 0.001$; 95% CI: 3.24–20.8). Bacterial infection was more common with increased trichiasis severity. In the conjunctival scarring case–control group, scarred eyes had slightly more bacterial infection (11%) than did normal control eyes (6%), although this was not significantly different (OR: 2.2; $P = 0.144$; 95% CI: 0.79–6.33).

CONCLUSIONS. Trichiasis is associated with increased risk of bacterial infection, and there may be a similar trend in eyes with conjunctival scarring. Bacterial infection of the conjunctiva is associated with inflammation, which may result in progressive scarring. Prospective studies are needed to determine the contribution of bacterial infection to disease progression. Bacterial infection probably also contributes to the development of corneal opacification. (*Invest Ophthalmol Vis Sci* 2007;48:4440–4444) DOI:10.1167/iovs.07-0315

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Blinding trachoma is a significant public health problem in some of the world’s poorest regions.¹ An estimated 8 million individuals are either blind or have severe visual impairment caused by trachoma. It is generally thought that repeated episodes of chronic follicular conjunctivitis (clinically active trachoma) caused by *Chlamydia trachomatis* infection drives a progressive inflammatory scarring process. Eventually, the eyelids turn inward (entropion), and the lashes come into contact with the eye (trichiasis), resulting in blinding corneal opacification.

Recent epidemiologic studies indicate that the scarring complications of trachoma continue to progress in regions such as The Gambia, where the prevalence of clinically active trachoma and chlamydial infection is now generally low after the introduction of control measures.^{2–7} The results of these studies suggest that other factors, in addition to *C. trachomatis* infection, may contribute to the progression of conjunctival scarring. Therefore, it is important to strengthen our understanding of the pathophysiology of trachoma, particularly in the advanced stages of the disease when blinding complications occur, to develop blindness prevention strategies.

A variety of factors may be involved in both the progression of conjunctival scarring and the development of corneal blindness.⁷ In addition to chlamydial infection these could include bacterial infection, ocular surface dryness, and a poorly regulated chronic inflammatory response. Recently, several studies from The Gambia have shown that bacteria can frequently be cultured from conjunctival swab samples in individuals with trichiasis.^{6–9} However, it is uncertain whether this represents a true increase in the risk of bacterial infection in eyes with trichiasis. It is also unknown whether people with trachomatous conjunctival scarring (without trichiasis) are at increased risk of bacterial infection. As a first step in investigating the possible contribution of bacterial infection to blinding trachoma it is necessary to determine whether individuals with trachomatous scarring or trichiasis have a significantly increased risk of bacterial conjunctival infection. Therefore, we conducted a case–control study in which individuals with trichiasis or conjunctival scarring alone were compared with normal control subjects.

METHODS

Ethical Permission

This study was approved by the Gambian Government/Medical Research Council Joint Ethics Committee and was conducted in accordance with the tenets of the Declaration of Helsinki. The nature of the study was explained to participants, and their consent was received before clinical assessment.

Study Participants

In this investigation, two case–control studies were conducted: one in 2002 and the other in 2003. In the first subjects with trachomatous trichiasis (TT) presenting for surgery were compared with normal control subjects. In the second study, subjects with trachomatous conjunctival scarring (TS) without trichiasis were compared with nor-

mal control subjects. In both studies, the control subjects were matched to cases for village of residence, sex, ethnicity, and age (within 10 years). In a few locations, it was not possible to find control subjects who met all the criteria. Under these circumstances, we recruited the person who most closely matched the criteria. One control was recruited for each case.

Trichiasis patients and their control subjects were recruited consecutively during the course of a systematic village by village trichiasis surgery program in Western Division, The Gambia. Patients with trichiasis requiring surgery were identified from the Gambian National Eye Care Programme (NECP) trachoma database, community ophthalmic nurse records, and village level screening. Normal control subjects were recruited by asking for a person of the same or neighboring household.

Individuals with trichomatous conjunctival scarring (without trichiasis) and their control subjects were recruited from 18 villages in the Upper Saloum District. The resident population of these villages was screened (90% coverage) for signs of trachoma during the course of other studies. Those in whom TS was diagnosed were invited to participate in this additional study.

Clinical Assessment

Subjects were assessed by an ophthalmologist or ophthalmic nurse experienced in the examination of patients with trachoma using 2.5× binocular loupes and a torch. Clinical signs were graded using the World Health Organization (WHO) trachoma grading system.¹⁰ Conjunctival inflammation was considered significant if there were prominent papillae and haziness of the tarsal blood vessels (P2 or P3). Lashes touching the cornea and other parts of the eye in primary position were counted separately. Evidence of epilation was noted: broken/regrowing lashes or sections of eyelid denuded of lashes. If evidence of epilation was found, the extent of the epilation was recorded as less than or more than half the length of the eyelid.

Bacteriologic samples were collected from the left eye of subjects with TS and all the control subjects. For subjects with TT, the eye with trichiasis was sampled. In subjects with bilateral TT, the eye with more lashes touching the globe was selected. The conjunctiva was anesthetized with preservative-free proxymetacaine 0.5% eye drops (Minims; Chauvin Pharmaceuticals, Romford, UK). An inferior fornix swab sample was collected and immediately placed into a sterile tube containing STGG (skimmed milk-tryptone-glycerol-glucose) broth for bacterial culture. A second swab was collected for *C. trachomatis* PCR from the upper tarsal conjunctival surface. Both sample types were kept on ice before transfer to -70°C or -20°C freezers, respectively, later the same day. Individuals with trichiasis were offered surgery the same day under the auspices of the NECP. Clinically significant conjunctival infection was treated with a course of topical antibiotic.

Tests for Infection

Bacteriologic samples were plated on blood agar (aerobic and anaerobic), McConkey agar (aerobic), gentamicin blood agar, and bacitracin chocolate agar. The gentamicin blood agar and the bacitracin chocolate agar were placed in a 5% carbon dioxide incubator. All plates were incubated at 37°C for 48 hours, and isolated organisms were identified using standard bacteriologic techniques. *Staphylococcus epidermidis* and *Bacillus* were excluded from the analysis, as they are considered commensal rather than pathogenic at this site. The second swab sample was tested for *C. trachomatis* DNA with a PCR-based assay (Amplicor CT/NG Test; Roche), used with previously described modifications.¹¹

Analysis

Data were analyzed in Stata 9 (StataCorp, LP, College Station, TX). This study used a matched case-control design. Therefore, conditional logistic regression was used to examine the relationship between disease state and other variables.

RESULTS

Trichiasis and Bacterial Infection

A total of 121 trichiasis (TT) case-control pairs were recruited. The individuals with TT and control subjects had comparable demographic profiles, although, due to the limited choice of control subjects in some locations, the control subjects tended to be slightly younger than the case subjects ($P = 0.005$), and in several instances a control subject of the same sex was not available ($P = 0.02$; Table 1). Subjects with trichiasis had an average of 12.2 (95% CI: 10.3–14.0) lashes touching the study eye before surgery (interquartile range: 4–16; total Range: 0 [epilating]–46). There were 72 individuals with bilateral trichiasis. In these cases the average number of lashes touching the eye not included in the study was 9.8 (95% CI: 7.5–12.5) which was not significantly different from the study eyes (paired two sided t -test: $P = 0.13$). Conjunctival inflammation was observed in many subjects with trichiasis (46%), but in none of the control subjects.

Bacterial pathogens were more frequently isolated from conjunctival swab samples collected from eyes with trichiasis (37.2%) than control subjects (7.4%; Table 2). Conditional logistic regression analysis confirmed the significance of this association (OR: 8.2; $P < 0.001$; 95% CI: 3.24–20.8). There was a significant increasing trend in the prevalence of bacterial infection with increasing burden of trichiasis (Table 2).

Corneal opacification (CC2/CC3 or CO) was associated with greater age, severe trichiasis, conjunctival inflammation, and bacterial infection (Table 3). Conjunctival inflammation was associated with bacterial infection (OR: 3.58; $P < 0.001$; 95% CI: 1.81–6.72). There was a significant increase in the prevalence of bacterial infection with increasing severity of corneal opacification: CC0 13%, CC1 35%, CC2 40% and CC3 81%; test for trend (Cuzak-Wilcoxon rank-sum test) $z = 6.68$, $P < 0.001$.

TABLE 1. A Comparison of Demographic and Clinical Characteristics of Individuals with Trichiasis and Their Matched Control Subjects

	Trichiasis Cases (n = 121)	Normal Controls (n = 121)
Demographics		
Females	95 (78)	79 (65)
Mean age (IQR)	55.7 (47–65)	50.7 (39–60)
Ethnicity		
Mandinka	57 (47.1)	58 (47.9)
Wolof	1 (0.8)	0 —
Jola	48 (39.7)	47 (38.9)
Fula	7 (5.8)	9 (7.4)
Manjago	7 (5.8)	7 (5.8)
Karoninka	1 (0.8)	0 —
Corneal opacity		
CC 0	56 (46.3)	121 (100)
CC 1	34 (28.1)	0 —
CC 2	15 (12.4)	0 —
CC 3	16 (13.2)	0 —
Trichiasis (number of lashes)		
0 (no trichiasis)	— —	121 (100)
0 (epilate)	5 (4.1)	— —
1–4	13 (10.7)	— —
5–9	47 (38.8)	— —
10–19	35 (28.9)	— —
20+	21 (17.4)	— —
Conjunctival inflammation (P2/P3)	56 (46.3)	0 (0)

Except where noted, the data are the number of subjects, with the percentage of the total in parentheses.

TABLE 2. Conjunctival Bacterial Infection by Clinical Status

	Trichiasis Cases	Normal Controls
Bacterial infection	45/121 (37.2)	9/121 (7.4)
Bacterial infection by trichiasis severity*		
Lashes (<i>n</i>)		
0 (epilate)	1/5 (20)	— —
1-4	3/13 (23)	— —
5-9	15/47 (32)	— —
10-19	12/35 (34)	— —
20+	14/21 (66)	— —

Data are the number of cases/total group, with the percentage of the total in parentheses.

* Test for trend (Cuzak-Wilcoxon rank-sum test) $z = 6.38$; $P < 0.001$.

Trachomatous Conjunctival Scarring and Bacterial Infection

A total of 117 trachomatous conjunctival scarring (TS or C2/C3) case-control pairs were recruited. The TS cases and control subjects had comparable demographic profiles, although due to the limited choice of control subjects in some locations these tended to be slightly younger than cases ($P = 0.051$) and in several instances a control of the same sex was unavailable ($P = 0.7$; Table 4). Bacterial pathogens were isolated more frequently from conjunctival swab samples from eyes with TS (11.1%) than control subjects (6.0%); however, this difference was not statistically significant (conditional logistic regression: OR: 2.2; $P = 0.144$; 95% CI: 0.79-6.33).

Bacterial Pathogens

A wide range of organisms were isolated from conjunctival swab samples in these studies (Table 5). Among trichiasis cases *Streptococcus pneumoniae* was the commonest organism accounting for 42% of isolates. This pattern of *S. pneumoniae* dominance was not seen in the TS cases or either of the control groups.

C. trachomatis Infection

Chlamydial PCR testing was conducted on all 121 trichiasis case-control pairs. Only one individual with trichiasis had detectable *C. trachomatis* DNA; no control subjects had detectable *C. trachomatis*. In the second study of trachomatous scarring without trichiasis it was possible to only test the first 44 case-control pairs by chlamydial PCR, all whom were negative.

DISCUSSION

These case-control studies investigated the association between conjunctival bacterial infection and trachomatous trichiasis and scarring. Eyes with established trichiasis were signifi-

TABLE 3. Multivariable Logistic Regression Model for Visually Disabling Corneal Opacification (CC2 or CC3) among All Trichiasis Cases and Controls

Variable	OR	95% CI	P
Age 60+ years	3.40	1.28-9.07	0.014
Severe trichiasis (10+ lashes)	6.82	2.43-19.2	<0.001
Severe conjunctival inflammation (P3)	4.78	1.37-16.7	0.014
Bacterial infection	3.96	1.48-10.6	0.006

TABLE 4. A Comparison of Demographic Characteristics and Conjunctival Bacterial Infection in Individuals with Conjunctival Scarring and Their Matched Control Subjects

	Scarring Cases (<i>n</i> = 117)	Normal Controls (<i>n</i> = 117)
Demographics		
Females	68 (58)	64 (55)
Mean age (IQR)	43.5 (29-58)	39.9 (29-51)
Ethnicity		
Mandinka	6 (5.1)	8 (6.8)
Wolof	90 (76.9)	88 (75.2)
Fula	21 (18.0)	21 (18.0)
Bacterial infection	13/117 (11.1)	7/117 (6.0)

Except where noted, the data are the number of subjects, with the percentage of the total in parentheses.

cantly more likely to have conjunctival bacterial infection than normal control subjects. This association strengthened with increasing disease severity, indicated by the number of lashes touching the eye. It is likely that trichiasis facilitates the introduction of bacteria into the conjunctival sac. Moreover, trichiasis may also compromise the resolution of a conjunctival bacterial infection by providing a "foreign body" surface for attachment. Eyes with tarsal conjunctival scarring without trichiasis tended to have more bacterial infection than control subjects; however, this difference was not statistically significant, possibly because of a limited sample size. It is possible that we were not able to culture all bacteria in these subjects, given the environmental and logistic constraints of working in rural Africa. However, these factors would have affected both groups equally, so would not bias any inferences that are drawn.

The central event in progressive trachomatous scarring, in common with other cicatricial conditions, is probably chronic inflammation. Conjunctival inflammation could be triggered or maintained by several factors including chlamydial and bacterial infection, ocular surface dryness, a smoldering, poorly regulated immune response and mechanical irritation from trichiasis. In this study *C. trachomatis* infection was very rarely detected. Therefore, it is unlikely to play a major role in driving disease progression in this environment at present. In contrast, bacterial infection was detected more frequently, particularly when trichiasis was present. Bacterial infection has previously been associated with conjunctival inflammation in eyes with trichiasis.^{7,9} However, a prospective study with frequent sampling for bacterial culture over several years is needed to determine the contribution of bacterial infection to disease progression.

Earlier studies have explored the relationship between bacterial infection and clinically active trachoma in children.¹²⁻¹⁷ It was thought that in regions where seasonal epidemics of bacterial conjunctivitis occurred bacterial infection may exacerbate the pathogenesis and transmission of trachoma.¹⁴ It was suggested that the severity of conjunctival inflammation from *C. trachomatis* infection may be increased by a coinfection with other bacteria. In addition, it was thought that person to person transmission of *C. trachomatis* may be enhanced by the presence of more copious ocular secretions. However, it is difficult to draw definite conclusions from these studies as most did not have normal control groups for comparison. In addition, direct comparison with more recent studies is complicated by the use of the older MacCallan trachoma grading system. The range of organisms cultured from these children appears to be quite similar to that found in adults with scarring complication of trachoma. The lower level of *Haemophilus*

TABLE 5. Pathological Conjunctival Bacteria Isolated in the TT and TS Case-Control Studies

	TT Case-Control		TS Case-Control	
	Case (n = 45)	Control (n = 9)	Case (n = 13)	Control (n = 7)
<i>S. pneumoniae</i>	19 (42)	1 (11)	1 (8)	
<i>S. viridans</i>		1 (11)	1 (8)	
<i>Streptococcus</i> group A			1 (8)	
<i>Streptococcus</i> group C	3 (7)	1 (11)		
<i>Streptococcus</i> group D			1 (8)	
<i>Streptococcus</i> spp.	8 (18)		1 (8)	1 (14)
<i>S. aureus</i>	5 (11)		4 (30)	2 (30)
<i>Pseudomonas aeruginosa</i>	1 (2)		1 (8)	1 (14)
<i>Moraxella</i>	3 (7)			
<i>H. influenzae</i>	1 (2)			
<i>Klebsiella</i>	2 (5)		1 (8)	1 (14)
<i>Neisseria</i> spp.	1 (2)			
<i>Coliform</i> spp.	1 (2)	5 (56)	2 (14)	1 (14)
<i>Salmonella</i>		1 (11)		1 (14)
<i>Enterobacter sakazakii</i>	1 (2)			

Data are the number of subjects, with the percentage of the total in parentheses.

influenzae found in this study (compared with earlier studies in children) may be due the very effective Hib vaccination program, which was introduced into The Gambia in 1997.¹⁸

Several animal models have been used to investigate the effect of coinfection with *Chlamydia* and other bacteria.¹⁹⁻²¹ These studies have given varied results. In a feline model of active trachoma using *Chlamydia psittaci*, coinfection with streptococci exacerbated the inflammatory disease.¹⁹ However, in a guinea pig model also using *C. psittaci*, additional infection with *Staphylococcus aureus* did not alter the course of the inclusion conjunctivitis.²⁰ The effect of secondary bacterial infection was also investigated in a monkey model of active trachoma, generated by repeated conjunctival inoculation with *C. trachomatis*. Monkeys infected with *Chlamydia* were no more susceptible to bacterial infection than were control monkeys, and the disease severity caused by a combined infection was similar to animals only inoculated with *Chlamydia*.²¹ In a separate series of experiments conducted on monkeys with conjunctival scarring produced by repeated exposure to *C. trachomatis*, a more severe and sustained inflammatory reaction was produced by bacterial infection, compared with control animals.²¹

Corneal opacification, the cause of blindness in trachoma, may result from various insults. Trichiasis is probably the single most important factor.^{7,9} However, new corneal opacification has been found to develop after the successful surgical treatment of trichiasis.⁹ This outcome suggests that other factors such as infection (bacterial and possibly chlamydial), ocular dryness, and chronic conjunctival inflammation may also contribute. Trichiasis traumatizes the corneal epithelium increasing vulnerability to secondary bacterial infection. Moreover, the increased prevalence of bacterial pathogens infecting the conjunctiva of eyes with trichiasis probably makes infection a frequent event.^{6,9} In the present study although severe trichiasis (10+ lashes touching the eye) was found to be the strongest risk factor for corneal disease, bacterial infection was also associated with increased risk of corneal opacification.

Overall, these data suggest that bacterial infection may be more important in the pathogenesis of late-stage trachoma than previously recognized. Eyes with increasing severity of disease appear to be more susceptible to bacteria. It is likely, although not proven, that the bacterial infection promotes conjunctival inflammation, contributing to progressive scar-

ring. In addition, bacterial infection is probably an important factor in the development of blinding corneal opacification in trachoma.

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