Bacterial Infection in Scarring Trachoma

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Purpose. To assess whether non-chlamydial bacterial infection is associated with trachomatous scarring in adults.

Methods. This was a case–control study of 360 cases with trachomatous scarring but without trichiasis, and 360 controls without scarring. All participants underwent clinical examination, and a swab was taken from the inferior conjunctival fornix. Samples were inoculated onto blood and chocolate agar later that day.

Results. Bacterial isolates were identified in 54.0% of cases compared with 34.6% of controls ($P < 0.001$). A multivariate logistic regression model adjusted for age and lack of education showed that scarring was associated with the presence of commensal organisms (odds ratio [OR], 1.46; 95% confidence interval [CI], 1.01–2.09) and was strongly associated with the presence of pathogenic organisms (OR, 4.08; 95% CI, 1.59–10.45). There was an increasing prevalence of all bacterial isolates with increasing severity of scarring ($P_{trend} < 0.001$).

Conclusions. Trachomatous scarring is strongly associated with non-chlamydial bacterial infection compared with controls. The role of such infection with regard to scarring progression should be investigated and may have important implications for trachoma control strategies and prevention of blindness. (*Invest Ophthalmol Vis Sci.* 2011;52:2181–2186) DOI: 10.1167/iovs.10-5829

Trachoma is the leading infectious cause of blindness worldwide. It is caused by infection with *Chlamydia trachomatis* and is characterized by inflammatory changes in the conjunctiva in children with subsequent conjunctival scarring, trichiasis, and blinding corneal opacity in adults. It is estimated that more than 1.3 million people are blind from the disease, 8.2 million have trichiasis, and 40 million have active disease.[1,2]

Over the past 30 years, because of improved living standards and the implementation of trachoma control strategies, there has been an encouraging downward trend in the global prevalence of people with active trachoma.[2–5] However, trachoma is still a public health problem in more than 50 countries with high levels of active disease where children are at future risk of scarring and blindness.[5] The number of people estimated to have trichiasis has shown little decline since 1991, suggesting that progressive conjunctival scarring can occur even when there has been a marked reduction in active disease and *C. trachomatis* infection and that those who already have conjunctival scarring are at risk of going on to blinding corneal opacity caused by trachoma.

While nearly all children in hyperendemic areas suffer repeated infection with *C. trachomatis*, it is unclear which factors drive the scarring process in the conjunctiva, why only a proportion of scarred subjects subsequently have trichiasis, and why only a proportion of these become blind. Severe inflammation and prolonged chlamydial infection appear to put children at increased risk of future scarring.[6,7] However, infection with *Chlamydia trachomatis* is only rarely found in adults, suggesting that this is not necessarily the only factor driving progressive scarring.[8–11] While the onset of trichiasis may be associated with chlamydial infection,[12] incident trichiasis has also been found to develop in a significant proportion of eyes in a cohort where the chlamydial infection rate was 1%.[12,13]

Chronic conjunctival inflammation is probably a key factor in the development of blinding trachoma.[6,7,13–15] An important element in maintaining this inflammatory state may be non-chlamydial bacterial infection. Previous studies have shown that (non-chlamydial) bacterial infection is found more frequently in patients with trichiasis and is associated with trichiasis recurrence after surgery.[8,10,15] Patients with trachomatous conjunctival scarring without trichiasis were also found to have an increased frequency of bacterial infection compared with controls, although this was not statistically significant, probably due to a limited sample size.[10] A recent study examining trichiasis patients 1 year after surgery found that, after adjustment for other factors, bacterial infection was significantly associated with elevated levels of interleukin-1β, matrix metalloproteinase-9, and the ratio of matrix metalloproteinase-1/tissue inhibitor metalloproteinase-1.[16] This finding suggests that bacterial infection may promote a proinflammatory and tissue remodelling response in the conjunctiva, possibly through innate immune mechanisms, which may be an important factor in the pathogenesis of trachomatous scarring and blindness.

The purpose of this study was to compare the frequency and type of non-chlamydial bacterial conjunctival infection between subjects with trachomatous scarring and controls. A strengthened understanding of the pathophysiology of trachomatous scarring and its progression will help in the assessment

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of current blindness prevention strategies and assist in the development of new interventions.

METHODS

Ethical Approval

This study adhered to the tenets of the Declaration of Helsinki. It was approved by the Tanzanian National Institute of Medical Research Ethics Committee, the Kilimanjaro Christian Medical Centre Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee. The study was explained to potential study subjects and written, informed consent was obtained before enrollment.

Subject Recruitment

This study was conducted in the Siha district of the Kilimanjaro region of northern Tanzania, in what was historically a single village. Two years before the study the area was divided into three administrative units, but these still form a single continuous geographic entity. Previous surveys of children in this village showed a moderate level of active trachoma. A survey conducted 6 months before the start of this study found a follicular trachoma (TF) prevalence rate of 18% among 1- to 9-year-olds. However, no children of the 43 randomly selected individuals from this village were positive for chlamydia infection by PCR (Amplicor; Roche Molecular Diagnostics, Mannheim, Germany) (Courtright P, personal communication, October 2010). A two-stage process was undertaken to identify suitable candidates for a case-control study. Initially, a census was made of the resident adult population (18 years or older). At the time of the enumeration, door-to-door visits were conducted, and available adults were screened for the presence of trachomatous conjunctival scarring. After participants with trichiasis or previous eyelid surgery were excluded, individuals with scarring were invited to join a related cohort study. Only those with more than minimal scarring (grade S1b or worse, see below) were included in the analysis of this case-control study. An equal number of village residents without scarring were invited to join as control subjects, frequency matched by ethnicity.

Clinical Examination

All subjects were examined by an ophthalmologist (VH) using ×2.5 loupes and a bright torch. Examinations were performed in a dark tent, ensuring standard conditions. The 1981 World Health Organization trachoma grading system[3] was used with some modification. The WHO system for grading conjunctival scarring does not have very objective definitions for "mild" or "moderate" scarring. Therefore, we developed a modified system for classifying tarsal conjunctival scarring (Table 1; example photographs shown in Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-5829/-/DCSupplemental).

TABLE 1. Tarsal Conjunctival Scarring Grading System

Grade	Definition*
S1	Scarring occupying <1/3 of the upper lid
S1a	One or more pinpoint scars and/or a single line of scarring less than 2 mm in length†
S1b	Multiples lines of scarring less than 2 mm in length
S1c	One or more lines/patches of scarring each 2 mm or more in length/maximal dimension
S2	Patches of scarring occupying in surface area ≥1/3 but <2/3 of the upper lid
3	Patches of scarring occupying in surface area ≥2/3 of the upper lid

* Upper lid, zones 2 and 3 of the everted upper lid.[3]

† 2 mm was chosen, as this is the approximate width of the lower lid margin, which is readily available for comparison.

Microbiology Samples and Analysis

The conjunctiva was anesthetized with preservative-free proxymetacaine 0.5% eye drops (Minims; Chauvin Pharmaceuticals, Montpellier, France). A rayon-tipped swab sample was collected from the inferior fornix and placed immediately into Amies charcoal transport medium (Sterilin, Caerphilly, UK) and kept at ambient temperature. Samples were inoculated onto blood and chocolate agar later the same day (rarely >6 hours, usually <5 hours from collection time) and incubated at 37°C for 48 hours. Culture isolates were identified by standard microbiologic techniques.

Sample Size and Data Analysis

This study was part of a larger series of related studies on the pathogenesis of trachomatous scarring with the sample size calculated to encompass these other components. The sample of 360 cases and 360 controls has >90% power to detect an association with non-chlamydial bacterial infection with an odds ratio of 2.5 when such infection is present in 6%[10] of control subjects.

Data were entered into a database (Access 2007; Microsoft, Redmond, WA) and analyzed (Stata 10.0; StataCorp LP, College Station, TX). The χ^2 test was used to determine strength of association for individual bacterial isolates or groups of isolates (commensal or pathogenic organisms) according to case-control status. A nonparametric test for trend was used to look at prevalence of bacterial culture by the ordered categories of scarring severity. Given the known association of trachomatous scarring with age, logistic regression models were used to estimate single-factor, age-adjusted, odds ratios (ORs) and 95% confidence intervals (CIs) for factors associated with the presence of scarring and inflammation. A multivariable logistic regression model was fitted, including age as an a priori factor, and other factors, if they were associated with scarring in the age-adjusted analysis and independently associated in the multivariable model ($P < 0.1$). Likelihood ratio tests were used to assess the strength of association of each factor with the outcome, and tests for nonlinearity were conducted to assess whether fitting age as a continuous variable provided an adequate fit to the data.

RESULTS

This village had an adult population of 3626 people at the time of the census, of whom 2418 (67%) were seen. Of those not seen, 711 (19.6%) were absent at the time of the census, despite two visits; 347 (9.6%) were temporarily resident elsewhere; and 150 (4.1%) refused examination. We excluded 36 (1.0%) due to the presence of trichiasis, previous eyelid surgery, or an inability to give informed consent. Of the remaining, 862 (23.8%) had trachomatous conjunctival scarring, and 1520 (41.9%) did not have scarring.

We recruited 360 cases with trachomatous conjunctival scarring and 360 control subjects without scarring. Baseline demographic characteristics are shown in Table 2. The majority of cases and controls were of Maasai ethnicity (77% of both groups) followed by Chagga ethnicity (11% of both groups). The controls were younger than the entire census population (mean age, 31.9 vs. 37.4 years; $P < 0.001$), which may cause an overestimation of the association of scarring with age in the study population. Odds of trachomatous scarring increased twofold with each 10-year increase in age ($P < 0.001$). After adjustment for age, lack of education was strongly associated with scarring.

Clinical findings are shown in Table 2. The scarring was mostly mild to moderate. Conjunctival inflammation (grades P2 and P3) was present in 25.5% of cases and in none of the controls. Follicles were found very infrequently in both cases (1.5%) and controls (0.3%).

Bacterial isolates were identified in 54% of cases, compared with 34% of controls ($P < 0.001$; Table 3). Coagulase negative

TABLE 2. Demographic and Clinical Characteristics of the Study Participants and Age-Adjusted Associations with Scarring

	Cases (n = 360)		Controls (n = 360)		Age-Adjusted Association with Scarring		
Parameter	***n***	**(%)**	***n***	**(%)**	**OR**	**95% CI**	***P***
Age groups, y							<0.001
18–25	29	(8.1)	117	(32.5)	1		
25–35	53	(14.7)	113	(31.4)	1.88	1.11–3.16	
35–45	65	(18.1)	85	(23.6)	3.13	1.87–5.26	
45–55	80	(22.2)	28	(7.8)	11.53	6.38–20.84	
55–65	57	(15.8)	9	(2.5)	28.75	12.36–66.87	
>65	76	(21.1)	8	(2.2)	34.07	15.28–75.95	
Age in years, mean (95% CI)	50.2	(48.3–2.0)	31.9	(30.7–3.1)	Trend 2.19*	(1.92–2.49)	<0.001
Sex							0.40
Female	219	(60.8)	241	(66.9)	1.00	—	
Male	141	(39.2)	119	(33.1)	1.17	0.81–1.68	
Formal education†							<0.001
None	250	(69.4)	166	(46.1)	1.00	—	
1–7 y	106	(29.4)	177	(49.2)	0.61	0.42–0.87	
>7 y	4	(1.1)	17	(4.7)	0.24	0.06–0.86	
BMI‡							0.55
Underweight	55	(15.4)	30	(8.5)	1.35	0.77–2.39	
Normal	257	(72.0)	284	(80.0)	1.00	—	
Overweight/obese	45	(12.6)	41	(11.6)	1.12	0.66–1.89	
Scarring grade§							
0	—	—	360	(100)			
1a	0	(0.0)	—	—			
1b	187	(51.9)	—	—			
1c	127	(35.3)	—	—			
2	33	(9.2)	—	—			
3	13	(3.6)	—	—			
Papillary inflammation grade							
P0	80	(22.2)	343	(95.3)			
P1	188	(52.2)	17	(4.7)			
P2	84	(23.3)	0	(0.0)			
P3	8	(2.2)	0	(0.0)			
Follicles							
F0	355	(98.6)	359	(99.7)			
F1	2	(0.6)	1	(0.3)			
F2	1	(0.3)	0	(0.0)			
F3	2	(0.6)	0	(0.0)			

* The result shown is the increase in the OR with each increase in age group category.
† Primary schooling in Tanzania is completed after 7 years.
‡ Categories from the National Institutes of Health.
§ All controls had no scarring (grade 0), and all cases had scarring grade 1b or higher.

staphylococci (CNS), *Corynebacterium* spp., *Streptococcus viridans*, and *Bacillus* spp. were designated as commensal organisms for the purposes of this analysis. Both pathogenic and commensal organisms were more prevalent in cases versus controls (pathogenic: 6.7% vs. 1.9%; OR, 4.90; 95% CI, 2.06–11.65; $P < 0.001$; commensal: 47.4% vs. 32.6%; OR, 2.09; 95% CI, 1.54–2.84; $P < 0.001$). There was an increasing prevalence of bacterial isolates (both commensal and pathogenic), with increasing severity of scarring ($P_{trend} < 0.001$, Table 4).

To assess the association between papillary inflammation and bacterial isolates among those with scarring, the cases were subdivided into either inflamed (P2 or P3) or noninflamed (P0 or P1). There were 92 (25.6%) inflamed cases and 268 (74.4%) noninflamed cases. A bacterial isolate (commensal or pathogenic) was cultured in 60 (65.2%) of the inflamed cases compared with 135 (50.4%) of the noninflamed cases (OR, 1.86; 95% CI, 1.14–3.04; $P = 0.01$). Commensal organisms were not significantly associated with inflammation, being found in 45 (48.9%) of the inflamed cases and 126 (47.0%) of the noninflamed ones (OR, 1.48; 95% CI, 0.89–2.48; $P = 0.13$). There was no evidence that individual commensal organisms were associated with inflammation. The presence of pathogenic organisms, however, was strongly associated with inflammation. They were detected in 15 (16.3%) of 92 of inflamed cases and 9 (3.4%) of 268 of the noninflamed (OR, 6.93; 95% CI, 2.79–17.24; $P < 0.001$).

Multivariable analyses showed that trachomatous scarring was independently associated with increasing age and lack of education (Table 5). Scarring was also associated with the presence of commensal organisms (adjusted OR, 1.47; 95% CI, 1.03–2.12) and was strongly associated with pathogenic organisms (adjusted OR, 4.08; 95% CI, 1.60–10.43).

DISCUSSION

This study showed, for the first time, that trachomatous scarring without trichiasis is strongly associated with non-chlamydial bacterial infection in cases compared with controls. Chlamydial infection itself is only rarely found in adults with scarring, and repeated infectious episodes with other bacteria may contribute to progressive scarring.

Several studies from The Gambia have examined the role of bacterial infection and inflammation in cicatricial trachoma, although most of these have been in patients with trichiasis. Bacterial infection was found before surgery in 30% of patients undergoing trichiasis surgery. Recurrent trichiasis at 12 months was associated with conjunctival inflammation and bacterial

TABLE 3. Bacterial Culture Results by Case-Control Status

	Cases (n = 360)		Controls (n = 360)		
	n	(%)	n	(%)	χ^2 test
Any isolate cultured	195	(54.2)	124	(34.4)	<0.001
Number of organisms cultured					
0	165	(45.8)	236	(65.6)	—
1	125	(34.7)	96	(26.7)	<0.001
2	66	(18.3)	24	(6.7)	<0.001
3	4	(1.1)	4	(1.1)	0.62
Type of isolate cultured*					
None	165	(45.8)	236	(65.6)	—
Commensal only	171	(47.5)	117	(32.5)	<0.001
Pathogenic +/− commensal	24	(6.7)	7	(1.9)	<0.001
Organisms					
CNS	110	(30.6)	69	(19.2)	<0.001
Corynebacterium spp.	89	(24.7)	42	(11.7)	<0.001
Viridans group streptococci	35	(9.7)	32	(8.9)	0.70
Bacillus spp.	8	(2.2)	8	(2.2)	1.0
Haemophilus influenzae, B	9	(2.5)	0	(0.0)	0.003
Streptococcus pneumoniae	7	(1.9)	1	(0.3)	0.03
Escherichia coli	3	(0.8)	0	(0.0)	0.08
Neisseria spp.	3	(0.8)	0	(0.0)	0.08
Staphylococcus aureus	2	(0.6)	0	(0.0)	0.16
Aeromonas hydrophilia	0	(0.0)	2	(0.6)	0.16
Fungus, mould	0	(0.0)	2	(0.6)	0.16
Gram negative rods (other)	1	(0.3)	1	(0.3)	1.0
Actinomyces	1	(0.3)	0	(0.0)	0.32
Enterobacter cloacae	1	(0.3)	0	(0.0)	0.32
Gram-negative rods, non-Lactose fermenting	1	(0.3)	0	(0.0)	0.32
Klebsiella spp.	0	(0.0)	1	(0.3)	0.32

* Commensal organisms include CNS, *Corynebacterium* spp., and *Viridans* group streptococci.

infection at 12 months.[8] Postoperative bacterial infection and conjunctival inflammation were also associated with recurrent trichiasis in another study in which examined patients were examined 3.5 years after surgery.[8,15] Bacterial infection and inflammation were found to be associated with major trichiasis (five or more lashes touching the globe) in a cohort study of Gambian patients with trichiasis who declined surgery; however, neither was significantly associated with progression in trichiasis after adjustment for other factors.[13] Finally, the first of two related case-control studies found that patients with trichiasis had an increased bacterial infection rate compared with controls and that infection was more common with increasing trichiasis severity.[10] In the second case-control study, while there was an increased infection rate in those with trachomatous scarring without trichiasis, this did not reach statistical significance (OR, 2.2; 95% CI 0.79–6.33; $P = 0.144$), probably because of a limited sample size. The current study, which has greater power, showed that scarring is associated with bacterial infection. Several clinical trials have investigated the effect of single-dose oral azithromycin after trichiasis surgery on TT recurrence. These have reported variable impact on the subsequent recurrence rate. It is plausible that at least part of the benefit of this intervention is attributable to the effect of this antibiotic on Gram-positive infection rather than on chlamydial infection alone.[8,17,18]

The results of studies on patients with trichiasis cannot necessarily be extrapolated to those with scarring alone, as the two groups are notably different. Trichiasis denotes lashes rubbing against the globe, causing mechanical damage and a persistent foreign body on the ocular surface. This effect provides a nidus for infection that may itself lead to an increased risk of infection and inflammation rather than the other way around. In this study we found that scarring without trichiasis is also associated with bacterial infection and inflammation. While cause and effect cannot be established with this study, we are currently observing a cohort of scarred subjects who are being assessed at regular intervals for infection and scarring progression.

Earlier studies have also examined bacterial culture rates in active trachoma, especially in relation to seasonal epidemics of

TABLE 4. Bacterial Culture Rates by Scarring Grade

	Conjunctival Scarring Grade										
	0 (n = 360)		1b (n = 187)		1c (n = 127)		2 (n = 33)		3 (n = 13)		Test for Trend
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	P
Any isolate cultured	124	(34.4)	84	(44.9)	78	(61.4)	23	(69.7)	10	(76.9)	<0.001
Type of isolate cultured											
None	236	(65.6)	103	(55.1)	49	(38.6)	10	(30.3)	3	(23.1)	
Commensal only	117	(32.5)	76	(40.6)	68	(53.5)	18	(54.6)	9	(69.2)	<0.001
Pathogenic +/− commensal	7	(1.9)	8	(4.3)	10	(7.9)	5	(15.2)	1	(7.7)	<0.001

TABLE 5. Multivariable Logistic Regression Model for Conjunctival Scarring

Risk Factor	OR	95% CI	*P*
Age group*	2.10	1.84–2.39	<0.001
Education			0.007
None	1.00	—	
≤7 years of education	0.61	0.43–0.88	
>7 years of education	0.29	0.08–1.07	
Type of bacterial organism cultured			0.002
None	1.00	—	
Commensal only	1.47	1.02–2.12	
Pathogenic +/− commensal	4.08	1.60–10.43	

* The result shows the increase in the OR with increasing age group category.

bacterial conjunctivitis.[19–25] However, most of these studies were in children without cicatricial stages of trachoma, did not contain control groups, and used older trachoma grading systems that are difficult to compare with those currently used. The two studies that did compare groups with and without trachoma showed little difference in the bacterial isolation rates between the two groups; however, these findings relate only to children with active disease.[24,25]

Monkey models of trachoma have also sought to elucidate the relationship between trachoma and bacterial infection. These have shown that bacterial co-infection in active disease did not result in more severe disease.[26] However, when bacteria were introduced into eyes with conjunctival scarring, a more marked and prolonged inflammatory reaction was produced compared with control animals.[26]

Another interesting question that our study raises is the role and definition of commensal organisms. Such organisms can act as part of the defensive mechanism of the ocular surface by preventing colonization and infection by more pathogenic bacteria.[27] However, deciding which organisms to categorize as a commensal can be a moot point. Previous studies have gone some way toward identifying organisms commonly found on the ocular surface of healthy eyes which generally behave in a non-pathogenic manner. These include CNS and *Corynebacteria*.[28–32] However, findings depend on various factors. Polymerase chain reaction with DNA sequencing, for example, detects a much broader range and higher frequency of organisms than does conventional bacterial culture.[31] The population being sampled is important, as many studies performed so far have been on subjects from developed areas. A recent survey from Sierra Leone of healthy eyes found a much higher proportion of isolates of bacteria usually thought of as pathogenic than previous studies, as well as fungi, the significance of which remains unknown.[32]

We found that one third of our control subjects had an organism cultured. This is on the lower end of the range compared with results of previous studies on the conjunctival flora, which showed culture rates of between 34% and 100%.[28–32] This discrepancy may be partly because our study was conducted in a remote, rural community and there was a short delay in getting the samples to the laboratory. However, the sample handling was identical between cases and controls, and we do not believe that any systematic bias resulted. We considered any increase in detection rates that may be achieved with plating the swabs directly onto culture media in the field would have been outweighed by higher contamination rates.

We included *Streptococcus viridans* and *Bacillus* as commensal organisms as well as CNS and *Corynebacteria*. *S. viridans* is a common oral commensal which is also frequently found in the ocular flora in trachoma endemic areas and does not appear to act in a pathogenic manner.[20,23,24] Poor dental hygiene in these areas may facilitate spread of the organism from the oral cavity to the eye, which are joined by a continuous mucosal surface. *Bacillus* was also included as a commensal organism, as a number of factors indicated that it was acting in a nonpathogenic manner. It was found equally in cases and controls; on all the occasions on which it was cultured, there was only mild growth and there was usually co-culture with other organisms; and it did not cause any clinically significant inflammation.

In our study, pathogens were significantly associated with inflammation while commensals (both individually and overall) were not. This result suggests that the scarred surface is more easily colonized by commensal organisms with little adverse effect, although a subclinical effect cannot be ruled out. The scarred surface also appears to be more prone to infection with pathogens that do cause inflammation and, perhaps, scarring progression.

Our study benefited from a prospective approach with standard clinical grading. There was minimal delay between taking the swabs and inoculating samples onto the culture medium. We also identified and adjusted for potential confounding factors. A potential confounder was the difference in age between cases and controls; however, bacterial infection remained significantly associated with scarring even after adjustment for age in a logistic regression model. Limitations of the study include not having a specific culture for fungi, which may have led to underestimation of their role. This study was conducted in an area mesoendemic for trachoma, and the level of scarring in the cases reflected this, being relatively mild, with most of the cases having less than one third of the upper lid scarred. This study, therefore, is applicable to the many other areas with a moderate level of trachoma. We found that the infection rate tended to increase with the level of scarring, suggesting that more bacterial infection would be found in communities with more severe scarring.

Chlamydial infection data are not available for this group of subjects. However, in this setting, we would expect the prevalence of infection with *C. trachomatis* in adults to be low, previous studies having suggested it the would probably be between 0% and 10%.[8–13]

In summary, trachomatous conjunctival scarring is associated with increased bacterial infection, the role of which warrants further investigation, especially with regard to scarring progression and the risk of blindness.

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