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Prevalence and Correlates of Trachomatous Scarring Severity among Adults in the Trachoma Hyper-endemic Amhara Region of Ethiopia

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Abstract

Prevalence and Correlates of Trachomatous Scarring Severity among Adults in the Trachoma Hyper-endemic Amhara Region of Ethiopia
By Jaymie Bromfield

Background In hyperendemic communities, conjunctival scarring can be observed in young adults and children due to frequent reinfection. Studies have shown that the prevalence of scarring increases with age, and women are more likely than men to develop severe signs of trachoma and related blindness. This study seeks to explore the factors and clinical features that contribute to various stages of scarring sequelae in an endemic area using a validated four-point severity scale.
Methods Left and right eye upper tarsal conjunctiva photographs, demographic information, and WASH indicators were collected in early 2017 as part of trachoma impact surveys in East Amhara, Ethiopia. Two graders assessed scarring severity in individuals aged 15 or older using Wolle et al.'s[26] scale (S1-S4). The highest score across conjunctiva was recorded to obtain person-level scarring severity. A third grader provided an adjudicating grade if needed.
Results Person-level scarring severity data was collected from 97.5% of adults. Scarring sequalae at every severity stage was observed among the youngest age group (15-19 years old). Age had a significant effect when comparing the odds of different scarring severity levels across age groups, with older individuals having higher odds of being in a higher severity level (include stat). Women were found to experience a greater prevalence of scarring at stages of severity compared to men (include stat). Additionally, living in a district with a trachoma follicular (TF) prevalence greater than 10% was significantly associated with an increased odds of scarring by over four-fold.
Conclusions The study identified increasing age, female gender, and residing in a district with a TF prevalence greater than 10% as independent correlating factors for increasing conjunctival scarring among adults in the region. These findings suggest that a considerable percentage of the population remains at risk of trichiasis development, and disparities in women and older age groups may be exacerbated by expected progression to come. The prevalence of trachomatous scarring, and its potential for progression to trichiasis, may prove a considerable barrier to achieving the elimination of trachoma as a public health problem.

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Literature Review

Introduction

Trachomatous trichiasis (TT) and corneal opacification, the blinding stages of trachoma, are the result of severe and irreversible conjunctival scarring. Typically, in endemic regions, frequent ocular infection with *Chlamydia trachomatis* begins in early childhood. Repeated Chlamydial infections first result in mild inflammation seen in the upper tarsal conjunctiva characterized by raised granules composed of germinal centers and surrounded by lymphoid tissues. This inflammation can become more severe, consisting of conjunctival thickening and obscurement of deep vessels across the tarsal surface. Within the simplified 5-scale WHO grading system, these stages are referred to as Trachomatous Inflammation – Follicular (TF) and – Intense (TI), respectively.[1] Most pre-validation surveillance focuses on two grades, named TF and TT, within the WHO simplified grading system to identify progress toward elimination as a public health problem. Over time, conjunctival scarring can develop as a result of vascular damage from severe inflammation. Trachomatous scarring (TS) is diagnosed at the point of visible scarring that interrupts or obscures the tarsal blood vessels.[1] Further scarring can lead to entropion, or inward turning of the eyelid until the eyelashes are misdirected and threaten the integrity of the conjunctiva and cornea. Without correction of inward-growing eyelashes, trauma to the cornea can lead to clouding, or opacification, that decreases vision. While the complications of trichiasis and corneal opacification normally arise in adulthood, hyperendemic communities have shown evidence of incident scarring in childhood in several previous longitudinal studies.[2-5]

Environmental Characteristics and Risk Factors of Trachoma

An association between facial cleanliness, due to the lack of ocular and nasal discharge, and reduced clinical signs of trachoma, as well as ocular *C. trachomatis* infection, has been observed in past research.[6-11] In an intervention to encourage face washing, Tanzanian children ages 1-7 years in villages that received a health education campaign focused on face washing were less likely to have severe trachoma at one year than in control villages.[6] Severe trachoma was defined as the presence of more than fifteen follicles or inflammation that caused complete obscuration of blood vessels in the tarsal plate. Children who sustained facial cleanliness at follow-up visits had a significantly lower rate of trachoma after one year in comparison to children that did not have or sustain a clean face.[6] Altherr et al[12] determined in an observational and cluster analysis that the mean clean face prevalence among 'hotspots' for TF was higher in comparison to non-hotspot districts. Few intervention studies have correlated a clean-face intervention with a reduction in signs of severe inflammation.[6,10] While facial cleanliness is considered a warranted measure of control against trachoma, ocular and nasal discharge may be related to both the cause and symptom of infection as trachoma can produce ocular discharge.[9]

Several cross-sectional and cohort studies have found an association between increased distance to the nearest water source and the prevalence of active infection[12-16] Altherr et al[12] determined that the prevalence and clustering of TF hot spots were negatively associated with household water access. However, the factors that contribute to water use for hygiene are often complex. Family size, crowding, socioeconomic indicators, and distance to the water source must be considered when assessing the association between water use and trachoma. As part of a 5-year longitudinal study of active *C. trachomatous* infection and trachoma, authors found a significant

variation in increased scarring in participants younger than 15 years old that was unexplained by age and sex alone, suggesting an impact of crowding or competing cause of morbidity among this cohort. [4] A case-control study conducted within a Gambian village observed significantly lower volumes of water associated with families diagnosed with trachoma, as well as a higher frequency of unclean faces.[17] In a sub-study of households with children aged 1-5 years located in Northern Tanzania, authors observed a decreased prevalence of active trachoma among households with a higher volume of water used for personal hygiene, regardless of the total volume.[18] Additionally, the full sample population observed increased prevalence with increasing average water collection duration (OR 2.25; CI 1.13–4.46).[18]

The presence of insecticide use and improved sanitation, such as latrines, is associated with a lower prevalence of trachoma. This is likely due to the reduction of a potential breeding ground for *Musca sorbens*, which are considered important vectors as they are frequently observed in clusters around the eyes of children and transmit C. trachomatis.[19] In a cluster-randomized control trial regarding fly control, the introduction of latrines was associated with a 30% average deduction in *M. Sorbens* surrounding the eyes of children and age-adjusted trachoma prevalence among the intervention group in comparison to the controls.[19]

Prevalence of Trachomatous Scarring (TS) in Amhara, Ethiopia

The percentage of adults, or individuals 15 years and older, with clinical signs encompassed by the WHO simplified definition of trachomatous scarring (TS) remains considerably high in the endemic region of Amhara, Ethiopia, despite the impact of the SAFE (Surgery, Antibiotics, Facial cleanliness, and Environmental improvement) strategy. Based on data collected from

population-based surveys of the 152 programmatic districts encompassing Amhara from 2010 to 2015, the regional prevalence of TS among adults was 12.6% (12.0-13.3%).[20] The prevalence of TS increased by age, with prevalence in individuals aged 56-60 and 61-65 observed at 22.4% and 24.2%, respectively. At the regional level, the difference in TS prevalence by sex was not statistically significant (males: 14.6%; females: 15.2%; p=0.73) although the range of difference by sex in each age group varied. Considerable difference in TS prevalence between the 10 administrative zones of Amhara was also observed, with the South Wollo zone presenting the lowest prevalence of TS among adults (7.2%, 6.3-8.3) in comparison the highest prevalence of TS within the South Gondar zone (23%, 20.8-25.4).

Other Characteristics and Risk Factors of Conjunctival Scarring

There is still uncertainty and limited research on the rate and driving forces of scarring incidence and progression from earlier clinical stages, particularly in hyperendemic and formerly endemic communities. It is hypothesized that individuals with a higher burden of active trachoma, infection, or both across their lifespan are at increased risk of scarring incidence, regardless of clinical signs of trachoma.[3] In one of the earliest prospective cohort studies of the relationship between active trachoma and scarring incidence in Tunisia, 17% of participants without scarring at baseline saw progression to severe scarring after a 14-year follow-up period as characterized by the detailed WHO grading system (C3).[3] In participants under 10 years old in Tanzania, no significant difference in frequency of scarring incidence was determined for children with constant infection (44.4%), constant trachoma (35%), or a combination of the two (31.2%).[3] The combined observation of severe, constant infection (defined as laboratory confirmation for at least 3 out of 5 visits within the first 18 months) and trachoma or constant infection only was a

high predictor of scarring incidence at 5 years of follow-up compared to children without constant severe trachoma or infection (OR: 3.07, p=0.0002).[3] However, in a four-year longitudinal study in Northern Tanzania, categorized proportions of *C. trachomatis* infection were only weakly associated with scarring progression.[21]

Thus, the incidence and progression of scarring may be independent of active trachoma and ongoing transmission. Within the early Tunisia study, a strong association was observed between the diagnosis of TI at baseline and incidence of TS (Risk ratio: 18) compared to a moderate association with the observance of TF (RR: 2.8). A prospective study in Tanzania examined the association between constant, severe trachoma, defined as 3 or more observations of TI within baseline year, and scarring incidence at 7 years in children ages 0 to 7.[22] West et al[2] observed higher odds of constant severe trachoma among individuals with scarring than those without. In addition to the presence of TI at baseline, strong associations were recorded independently with females and increased age. Within a previously hyperendemic community with a low incidence of scarring among children 1-9 years old, the risk of scarring was greater among females rather than males.[23] Additionally, more than 20% of children with scarring at baseline observed progression. Within the Northern Tanzania cohort study,[21] a strong association was observed between scarring progression and both TF and "significant papillary inflammation" (TP), equating to the diagnosis of TF and/or TI, after adjustments for age and sex at baseline.[21] It is also important to note that a greater risk of scarring progression was also seen among females in the study.[21] However, multivariate models of children without scarring at baseline showed only a strong association of scarring incidence after adjustments for TF alone, active infection, sex, and age at baseline.[21] Only a slight association for the female sex category was determined using the

multivariate model.[21] No association between scarring progression and TF nor active infection after retaining covariates was observed, suggesting that progression may be mediated by TP.[21] Much of the literature thus far regarding scarring progression and incidence has focused on the youth population. In a population-based study of women ages 15 and older from central Tanzania, scarring incidence increased remarkably between age groups, ranging from 3.1% in women 15-19 years old to 14.3% in women 55-59 years old.[5] From a sample of women in Kongwa, Tanzania, indicators of high socioeconomic status, such as bike ownership and head of household education, were significantly associated with decreased scarring incidence (OR[bikes]: 0.411; OR[HOH education]: 0.242).[24] In a two-year longitudinal study in Tanzania and Ethiopia of adults with some evidence of conjunctival scarring at enrollment,[25] a strong relationship was observed between scarring progression and a higher observation of severe inflammation events in the absence of active infection (5.93, 3.31–10.6; $p<0.001$). More than 25% of adults observed scarring progression within the study period, despite infrequent detection of *C. trachomatis*.[25] Thus, conjunctival inflammation is a probable driver of late-stage trachoma including scarring. [25] In Kongwa, an area of Tanzania previously studied[4] that recently transitioned to a level of low trachoma prevalence, authors Wolle et al[26] found a comparable rate of scarring progression. This suggests that progression may still occur regardless of ongoing transmission status, insinuating that formerly hyperendemic communities may need to prioritize the mitigation of late stages of the disease to achieve the overall elimination of trachoma.

Validity and Reliability of Photographic Grading for Trachoma Diagnosis

The validity of in-field trachoma grading assessments conducted by trainees is normally certified using a kappa statistic of agreement with experienced graders. This certification and

standardization process can be conducted live or using a curated sample of photographs that eliminates borderline or difficult clinical grades where experienced graders do not agree.[27,28] Photographs collected of the upper tarsal conjunctiva during programmatic surveys have also been utilized to ensure adequate competence of in-field graders and provide future training. As the global prevalence of trachoma declines, authors have not only investigated clinical status by still photographs alone, [6,29] but the potential to replace field graders as a cost-effective alternative.[30-33] Much of the literature thus far is focused upon agreement between in-field and photographic graders of clinical signs of TF and TI. The literature regarding the reliability of photographic grading to validate or assess TS is limited.[30,32] Whitson et al[30] determined a lower percent agreement for TS compared to that of TF and TI. West and Taylor[32] found a comparable reliability percentage for photographic grading in a relatively small sample size.

The diminishing number of children with TF available for assessment and the resulting decrease in preliminary clinical signs of trachoma are making it increasingly challenging to train graders to the same high level of expertise as before and to sustain an adequate workforce of trained personnel within the country. Collection of masked conjunctiva images also eliminates any potential for bias, as the village location, the person (such as facial cleanliness), or intervention status (if applicable) cannot likely be identified.[19,34] From a low-prevalence setting,[32] photograph and in-field graded assessments of TF and TS showed considerable agreement (kappa=0.71,0.32 respectively), while TI indicated a good but widened range of agreement (kappa: 0.74; 0.52-0.92). Roper and Taylor[33] reported a significantly lower agreement between in-field and photographic grading for TI at 0.37, although the results of TF agreement were comparable to that of West and Taylor[32], Ramadhani et al,[21] Gebresillasie et al.,[35] and Whitson et al[30] at a median kappa of 0.71.[31] Solomon et al[36] observed one of the lowest agreements between three

photograph graders and the in-field grader. With an exclusion criterion of only photographs with inadequate focus, poor lighting, limited visualization of the central tarsal place, and insufficient eyelid eversion, all other photograph pairs were considered as well as disagreements upon gradability. The observed agreement between in-field and photograph graders was 0.51 and 0.44 for TI and TF, respectively. [36] Whitson et al[30] also reported considerably low agreement between in-field and photographic graders for TI and TS at κ=0.32 and 0.22, respectively. Previous literature, including Solomon et al,[36] have considered but ruled out the utility to exclude ungradable photographs from agreement calculations. Instead, the limitations in photographic grading previously cited, such as the inability to see the conjunctiva in a three-dimensional way with flexibility in view, warrant standardized guidelines for trachoma photography.[28,36]

Conclusions

The purpose of this literature review is to analyze the current characteristics and risk factors associated with trachoma and conjunctival scarring, the state of trachomatous scarring in Amhara, and the reliability of photograph grading for trachoma surveillance. While TF and TT prevalence are the indicators by which trachoma elimination is examined, evidence shows that scarring may be a useful predictor for TT incidence and may warrant consideration in hyperendemic communities which remain at high risk of scarring progression. Given limitations expected in adequate, efficient grader training as countries near elimination thresholds, trachoma control programs may benefit from the use of photographic grading to supplement or replace in-field surveillance and inform surgery uptake and planning for years to come.

Introduction

Trachoma, the leading cause of infectious blindness worldwide, is caused by repeated ocular infection of the bacterium *C. trachomatis.*[37] It is normally assessed by the WHO simplified grading system.[1] Active trachoma, or the presence of clinical signs of TF or TI, is commonly observed in childhood due to the transmission of *C. trachomatis* occurring frequently between children. [37] Further clinical stages of the disease, such as TT, corneal opacity, and visual impairment, are correlated with increasing age and are unlikely to occur before adulthood. Mathematical models of trachoma indicate that 151 conjunctival C. trachomatis infections are required in one's lifespan to develop TT later on in life.[38] However, those living in areas of high endemicity are likely to experience disease sequelae earlier in their life due to the speed at which they reach this threshold number of infections. Children who later develop TT are likely to experience reinfection more than once per month during childhood.[38]

The criteria provided by the World Health Organization to eliminate trachoma as a public health problem includes: i) a TF prevalence below 5% among children 1-9, ii) a TT prevalence "unknown to the health system" below 0.2% among adults older than 15 years of age, and iii) evidence of a health system capable of identifying and managing TT. These criteria must be met during two population-based surveys, historically conducted through field conjunctival examinations by certified graders.

Trachomatous scarring is a result of a Th2-cell-mediated immune response and is characterized by a chronic proinflammatory response that causes epithelial damage and proliferation, followed by scar formation.[37,39] Unlike active trachoma, scarring is considered to be the first irreversible stage of the disease, and some individuals without intervention may experience progression to

entropion of the eyelid, TT, CO, or eventual blindness. Trachomatous scarring is generally noted to occur in adulthood, although studies within communities of high endemicity have observed scarring in children likely due to the frequency of reinfection and rates of active trachoma.[2-4,20,40] Baseline scarring, as well as scarring severity, have been strongly linked to the risk of TT development.[41,42] Given the Amhara region's extensive history of trachoma exposure, a considerable percentage of the population is at risk of trichiasis development which may prove a considerable barrier to achieving the elimination of trachoma as a public health problem. Thus, this study aimed to quantify the burden of scarring among adults living in endemic communities of the region.

Among individuals in a region-wide population-based study of Amhara, trachomatous scarring prevalence increased significantly with age and more than 25% of individuals aged 60-64 were prevalent with trachomatous scarring.[20] Several prevalence studies have also shown that women are more likely to develop signs of severe trachoma and trachoma-related blindness than their male counterparts. In a meta-analysis of more than 12 countries, the odds of trichiasis among women were 1.82 times the odds among men (2.36,-6.19).[43] Women are estimated to account for 60-80% of all trichiasis cases.[44-47] Previous authors have demonstrated that conjunctival scarring continues to progress regardless of clinical signs of active trachoma.[24,48,49] Longitudinal studies have linked chronic conjunctival inflammation to scarring progression, while current infection with *C. trachomatis* has only sporadically been associated with trachomatous scarring.[2,3,22,23] It is evident that there are several contributing factors to the progression and development of scarring.

Conjunctival photographs have been used to train and assess field grader quality in conjunction with in-field validity examinations. Recently, authors have proposed the potential for conjunctival photographs to replace in-field examinations as part of large-scale programmatic surveys as more districts and countries continue to meet the elimination threshold for TF and clinical signs of preliminary disease stages diminish from communities and reduce training capabilities.[30,50] Few studies have demonstrated the potential for photographic grading to identify rates and associations of scarring progression and TT incidence.[2,48] Thus, the purpose of this study was to elucidate the potential for the progression of scarring and incident TT in adults of the Amhara region by understanding the prevalence, magnitude, and correlates of conjunctival scarring stages using field-collected conjunctival photographs and a validated four-point scale.[4,22,23,26]

Methods

Ethical Considerations Survey protocols was approved by Emory University Institution Review Board and the Amhara National Regional State Health Bureau (#079-2006). This survey was conducted in full compliance with the Declaration of Helsinki as informed oral consent among all participants was collected in 2017 with IRB approval, due to low literacy rates within the population.[30]

Study Area Amhara is a trachoma-endemic region with a high burden of trachomatous scarring and trichiasis.[20,51,52] Based on 152 population-based studies within Amhara published in 2021,[20] the 5 zones encompassing the sub-region of East Amhara had at least one district with a TS prevalence ≥ 15 %, with Waghemra zone having the heaviest burdens of scarring prevalence

across the region. As of 2022, no district of East Amhara has reached below the elimination threshold for TT.[53]

Data Collection This analysis used conjunctival photographs collected in conjunction with trachoma impact surveys conducted in January and February of 2017 within East Amhara. The data collection methods have been extensively reported previously.[30,51,52] To summarize, the population-based survey used multistage cluster random sampling methodologies to select villages (clusters) in the first stage and households in the second stage to estimate trachoma outcomes 3-8 years following SAFE strategy implementation. Each participant or their consenting guardian provided demographic information on their sex and age in addition to household-level data on WASH indicators, such as latrine use, the presence of an improved water source, and distance to and from the water source. Additionally, in-field observations of the trachoma signs including TF, TS, TT, and CO were made by trained and certified graders. Survey recorders collected data from participants using tablets compatible with an Open Data Kit application.[54]

Photography Photographic methods including camera specifics and photographer training have been published previously.[30] Briefly, one trained and certified photographer used a DLSR camera (Canon EOS 60D) to photograph all survey participants examined in 10 survey clusters (1 in each of 10 survey-eligible districts). The photographer was assigned to 1 of 4 field graders for each cluster-dependent on survey logistics for that given day. The photographer took at least two photographs per eye. Additional photographs were taken at the discretion of the photographer due to quality concerns.

Photographic Grading Photographs were graded for scarring severity based on a four-point validated scale by Wolle et al.[4], ranging from S1-S4 with subcategories of level S3 (see Table 1). Prior to grading, graders attended a 3-day training at the Wilmer Eye Institute of Johns Hopkins Hospital, Baltimore, USA. Training included lectures, analysis of conjunctival photographs, and practice examinations, and culminated with a final certification examination where trainees' grades were compared to those of two professionals (SKW and MW) with extensive experience in trachoma grading. Trainees completed 3 practice examinations with 150 photographs prior to the final examination set of 50 individual eyes. Graders passed the final examination and were considered certified with a kappa score comparable to two experienced professionals ($k>0.7$).

Eye-level scarring severity data collection from each grader was conducted in R (R Foundation for Statistical Computing, Vienna, Austria). Two independent graders assessed two photographs of each conjunctiva concurrently, of adult participants (15 years or older) at adequate magnification between November 2022 and January 2023. Other environmental conditions for grading include dim room lighting, maximum screen brightness, and full resolution of the photograph encompassing the screen. Each grader was masked to all collected demographic information and the eye-level identification number of each photo set.

The adjudication and aggregation process included the grading program which selected all eligible conjunctival identification numbers that have been graded by the two individuals. Concordant eye-level severity data across graders were automatically finalized and aggregated to the person- level by selecting the highest severity score of both eyes. Image file names corresponding to the discordant data across graders at the eye-level were then sent to a single

experienced trachoma grader (SKW) for adjudication. Cases where eye-level scarring severity was concordant between the experienced grader and one primary examiner were then finalized and aggregated to the person-level. Although rare, cases of discordant grades between both primary graders and the experienced professional or a large discrepancy (greater than one level difference) across the eye-level scores of an individual were then openly adjudicated by group discussion and finalized by the experienced grader on an as-needed basis.[55] While 245 person-level photograph sets required adjudication by the experienced grader, less than 50 prompted open adjudication.

Figure 1
Definition of conjunctival scarring clinical signs

Stage	Description
S1	One or more lines of scarring at least 3 mm in length and some stellate scars, but total scarring occupying less than one-eighth of the upper eyelid
S2	Multiple lines or patches of scarring obscuring at least one-eighth of the upper eyelid, but total scarring occupying less than one-third of the upper eyelid
S3	Scarring of at least one-third of the upper lid with clear conjunctiva between, but total scarring occupying less than 90% of the upper eyelid
S3 – A	Scarring of at least one-third of the upper lid with clear conjunctiva between, but total scarring occupying less than 50% of the upper eyelid
S3 – B	Total scarring occupying between 50-90% of the upper eyelid
S4	Scarring occupying more than 90% of the conjunctiva

Statistical Analysis Survey and scarring severity data analysis were conducted in R. All adults with successfully graded individuals were included in the descriptive analysis. The subsequent

analysis excluded individuals with missing demographic, in-field examination, or WASH characteristic data in addition to poor photograph quality. Distributions of scarring prevalence with exact 95% confidence intervals across potential correlates were graphed to inform any further analyses. Evidence for clustering by cluster and household was considered using individual random effects modeling techniques and the resulting interclass correlation coefficients prior to exploratory ordinal logistic regression analyses of each possible correlate of scarring severity. District prevalence was used as a proxy for continuing *C. trachomatis* transmission, and was split as >10%, and <10%, as evidence suggests that *C. trachomatis* infection is rare in Amhara once TF is below 10%.[56,57] The initial model was created using a mixed-methods ordinal logistic regression and the *ordinal* package in R to model the fixed effects of age while adjusting for cluster-level clustering. The final multivariable model was determined by a backward elimination approach to variables associated with scarring level at a p-value<0.2.

Results

Within the 10 study clusters, we collected photographs from 748 individuals ages 15 years and older (range per cluster: 44-120 individuals). Of the 748 individuals photographed, 729 (97.5%) were able to be graded for scarring severity, with 19 individuals excluded from subsequent analysis due to poor photograph quality. The mean age of individuals with graded photographs was 38 years and 62.8% were female, with 50% of the participants falling within the ages 27-54 years old (Table 2). The percentage of participants reporting access to an improved water source was 74.6%, and those with access to a latrine was 35.9% (Table 2). Thirty-one (4.3%) CO or TT cases were identified amongst study participants, of which 25 (80.6%) were identified with

severe (S4) scarring which indicates scarring obscuring more than 90% of the upper tarsal conjunctiva. The proportion of individuals with any conjunctival scarring was notably high, with a prevalence of 59.1% (95% CI: 55.4, 62.8). Most cases were classified as the most severe level of scarring (S4), accounting for 19.9% of cases, in contrast to the prevalence of S3 (11%), S2 (8%), and S1 (18.8%). Overall, it was found that 384 of 1453 photograph pairs (i.e., eye-level photographs) required adjudication. Of these pairs, 24.1% were classified as S1 following adjudication.

Results from age-specific distributions of scarring severity showed that scarring sequelae at every stage were observed among the youngest age group (15-19 years old) (Figure 1). Overall, most scarring cases (19.9%) fell within the highest level of scarring category (S4), compared to the prevalence of S3-A and B (11%), S2 (8%), and S1 (18.8%) (Table 2). More than 70% of older adults (≥60 years old) were prevalent for any stage of scarring (95% CI: 61.9, 78.1) (Table 2). 60–74-year old's also experienced a greater burden of severe scarring (S4 prevalence: 32.6%; 95% CI: 24.7, 41.3) than their 15-19-year-old counterparts (6.2%; 95% CI: 2.0, 13.8). According to the results of an unadjusted logistic regression model investigating the relationship between age and scarring severity, age was found to have a significant impact when comparing the odds of various scarring severity levels across all age groups (Table 3). Compared to the reference group (age group 15-19), participants in the 60-75 age group had higher odds of being in a higher severity level (OR = 2.7; 95% CI:1.35, 4.1), as did those in the 50-60 (OR = 1.8; 95% CI: 1.0, 3.2) age group ($p<0.01$) (Table 3).

Women are also observed to experience a greater prevalence of scarring at every stage of severity compared to men (Figure 2). While early stages of scarring were comparable in men and women, women were observed with a significant disparity in the more severe clinical stages of scarring, such as the S4 and S3 categories. The prevalence of severe scarring (S4) among women was 1.74 times the prevalence of scarring among men (CI: 1.1, 2.7, $p<0.01$) (Table 2). According to the findings from the unadjusted logistic regression model exploring the relationship between gender and scarring severity, there was a statistically significant difference in the odds of being in a higher disease severity category between males and females ($p<0.001$). Among those with severe disease, the odds of being female were 1.74 times higher than the odds of severe disease among males (95% CI: 1.3, 2.3) (Table 3). Furthermore, the association between sex and scarring severity did not suggest statistical interaction by age, with an odds ratio of 0.9 ($p=0.3$).

Several household factors were analyzed for association with scarring severity (S1, S2, S3, and S4) (Table 4). Individuals residing in households with reported latrine access had significantly lower odds of increased scarring compared to those without reported latrine access ($p<0.01$) (Table 4), with the odds of being in a higher scarring level being 0.63 (95% CI: 0.5, 0.8) times lower in the reported latrine access group than in the group reporting no latrine access (Table 4). Individuals residing in households with reported latrine access had significantly lower odds of increased scarring compared to those without reported latrine access ($p<0.01$) (Table 4), with the odds of being in a higher scarring level being 0.63 (95% CI: 0.5, 0.8) times lower in the reported latrine access group than in the group reporting no latrine access (Table 4).

Woreda (district) TF prevalence was observed to be a significant correlate of scarring severity. The average woreda-level TF prevalence at the time of this study was 9.7%, ranging from 2.0% in Ambassel to 55.3% in Dahana. Residence in a woreda with a TF prevalence greater than 10% increased the odds of scarring by over four-fold (OR: 4.3; 95% CI: 3.2, 5.8) compared to the corresponding odds among districts with a TF prevalence less than 10% (Table 4). Clustering of scarring prevalence was investigated with little found at the household level (ICC=0.004). However, the ICC for scarring severity of participants within the same cluster was 0.4 and indicative of notable variability that should be considered in future analyses.

The mixed-effects ordinal logistic regression model included all participants with demographic information, aggregating S3 subcategories (n=707), and employed a backward stepwise approach to reach parsimony. The approach identified sex, age group, and woreda-level TF prevalence as important factors for scarring severity (Table 5). After adjusting for sex, TF prevalence, and within-district variability, individuals between ages 60-75 years old had higher odds of increased scarring severity compared to those of the reference group (i.e., 15–19-year old's) (Table 5). Individuals within districts with TF prevalence greater than 10% had significantly higher odds of being in a higher scarring severity level compared to those in districts with TF prevalence below 10% (OR = 6.0; 95% CI: 1.3, 27.3; $p<0.05$) (Table 5). The odds of higher scarring severity, among females was 1.8 (95% CI: 1.3, 2.4; $p<0.0001$) times the corresponding odds among males adjusting for other factors (Table 5).

Discussion

Key findings Despite significant uptake of the SAFE strategy in Amhara since 2010 and reductions in clinical signs of TF, conjunctival scarring remains highly prevalent in the region and much of its older population remains at risk of TT and CO development. Hence, this study described the magnitude of conjunctival scarring using a 4-level disease severity grading system and tested the association of age and gender with conjunctival scarring among adults in this historically endemic region. In this sample, most adults observed some level of conjunctival scarring. When present, scarring was generally severe, with more than a third comprising the most severe category (S4) where >90% of the tarsus is interrupted by scarring. This study revealed that increasing age, female gender, and residing in a district with a TF prevalence > 10% were all independently related to increasing conjunctival scarring among adults.

Interpretation of findings A strong, negative association was determined between sex and scarring severity, which remained after controlling for age and district-level TF prevalence. Additionally, the prevalence of severe scarring (S4) among females and males was 23.5% (95% CI: 19.6, 27.7%) and 15% (95% CI: 10.9, 19.9%), respectively ($p<0.01$). This reinforces the results of earlier studies that demonstrated a higher likelihood of female adults developing severe scarring or blindness as a result of trachoma.[43,45,58-60] It is probable that women are more susceptible to developing severe trachoma-related complications due to their societal responsibilities in childcare, which creates a greater mode of exposure to active trachoma and a greater risk of recurrent infection.[61] The increased likelihood of women developing severe sequelae due to higher trachoma exposure and an increased risk of recurrent infection is likely a result of their societal roles in childcare.[61] Wolle et al[26] previously reported that the progression of scarring may be correlated to age as a result of biological factors that exacerbate the disease in

young and middle-aged women.[55] As women have been observed with higher incidence and severity of autoimmune conditions during their reproductive years and pregnancy, and scarring due to *C. trachomatis* infection causes an inflammatory immune response leading to conjunctival scarring, there could be a biological similarity within autoimmune diseases and scarring.[62] Therefore, the association between age and scarring observed in women may be due to biological factors that result in a more severe inflammatory immune response during their reproductive years, which then subsides and results in a slower rate of scarring progression after menopause. In our study, 34% of women ages 20-29 years were classified as having severe disease, compared to 15% of men with severe disease. Although the interaction term between age and sex in the relationship with increased scarring was not found to be statistically significant it is still biologically plausible that these two correlates may act synergistically to increase the risk of developing severe scarring sequelae.

The severity of scarring increased with age, as over a quarter of individuals aged 60 were observed with clinical signs of severe scarring. However, 15–19-year old's in this trachoma-endemic environment were found to have detectable levels of conjunctival scarring, and more than a quarter of individuals in this age group had scarring at or above S2 (Figure 1). This observation supports the notion that in communities where trachoma is highly endemic, scarring begins to form during childhood and adolescence, then continues to worsen as individuals age.[20] In a previous prospective cohort study in Amhara, 23% of adults with scarring exhibited progression between enrollment and 24 months.[25] A previously mentioned population-based study within Amhara also demonstrated that scarring prevalence increased significantly with age, ranging from 1.1% among children (1-9 years old) to 22.4 % among adults ages 56 to 60. The

progression of scarring at younger ages among endemic communities is expected to be a result of both cumulating *C. trachomatis* infection and recurrent inflammation. Given Amhara's extensive history with persistent infection, the region is likely to observe considerable scarring progression and incident TT long after elimination goals of TF is reached.[51,52,63]

The mixed effects logistic regression model adjusted for age and sex showed that increasing scarring severity was associated with TF prevalence, as a proxy for ongoing *C. trachomatis* transmission, at or above 10%. While A, F, and E interventions have occurred in these districts for more than 8 years, our results and previous studies have shown that many districts still observe a prevalence above 10% and the WHO trachoma elimination threshold of 5%.[51] While several investigations have clearly established the relationship between active trachoma (TF/TI) and recurrent infection,[57,64,65] the direct association of scarring progression and *C. trachomatis* infection is mixed.

The relationship between *C. trachomatis* infection and scarring incidence varies greatly between communities of varied endemicity. In Amhara, districts with a TF >10% are more likely to have detectable *C. trachomatis* infection,[56] and greater evidence of *C. trachomatis* transmission.[57] However, only 60% of adults with the presence of severe scarring lived within a district TF prevalence >10%, compared to those living within low-prevalence districts. Scarring incidence is reportedly lower in hypo- and formerly hyper- versus hyper-endemic settings, likely a result of reduced community transmission.[22] However, scarring and further disease progression have been observed in communities that are no longer endemic.[22,66] Others have reported significant risks of scarring incidence in adults and children in hyper-endemic communities associated with

constant infection, or detection of infection at multiple time points, and severe trachoma, defined as follicular trachoma greater than 10 follicles or TI or both.[2,3] Compared to those with sporadic infection or milder trachoma, 5-year scarring incidence was comparable among children who had constant severe trachoma, constant infection only, and both (35%, 44%, and 31%, respectively; $p < 0.001$).[3] Such large discrepancies between endemic groups have led to an exploration of genetic and epigenetic factors that differentiate responses to *C. trachomatis* and the development of inflammatory responses.[21,25,67,68] In particular, differential host response profiles have been reported between scarred individuals with and without inflammation.[68] In a conjunctival gene expression analysis conducted by Burton et al,[25] scarring progression was significantly associated with recurrent episodes of conjunctival inflammation. Additionally, the results of this study suggest the potential for biological susceptibility to the prolonged inflammatory episodes that characterize scarring as altered *CTGF*, a factor with pro-fibrotic properties and responsible for some functions of extracellular proteins, was also associated with inflammation.[25] In a longitudinal study of the incidence and progression of existing scarring of children in northern Tanzania, Ramdhani et al[21] found slim unadjusted associations between active infection or TF and scarring progression. Following crude analysis, parsimonious multivariate models excluded both variables when adjusting for conjunctival papillary inflammation (TP).[21] The available evidence implies factors other than *C. trachomatis* infection, which contributes to the development of inflammation, may continue to progress conjunctival scarring in communities of low current endemicity. In hyperendemic communities, the relationship between infection and TF with the progression of scarring is mediated by mechanisms that involve the inflammatory response.

Eastern Amhara represents a considerable population at risk of TT incidence, given the magnitude of severe scarring concentrated among women and older age groups within this study. Munoz et al[5] have modeled age-stratified incidence rates among women with and without scarring in the hyperendemic Kongwa district of Tanzania, embedded into a population-based sample for trachoma studies.[69] Among those with scarring, authors reported an observable difference in the incidence rate estimated between age groups of the population and solely those with scarring present.[5] Individuals with scarring had a 5-year incidence rate of trichiasis ranging from 3.2% among those aged 15-19 to 15.1% among those aged 50-59, compared to the population's incidence range of 0.3% among 15–19-year-olds to 6.4% among those 50-59 years old.[5] Munoz et al[41] also reported the incidence rate of trichiasis among 6 of 11 villages included in this sample, where 523 individuals presented clinical signs of TS at baseline. A seven-year incidence rate of 1.3% was determined among those with evidence of scarring upon enrollment compared to 0.1% among healthy individuals.[41] At the time of surveillance, most trichiasis cases within the current study were also observed with severe scarring sequelae constituting more than 90% of the upper tarsal conjunctiva, providing further evidence of the positive relationship between severe scarring and trichiasis development. If we compare the definition of TS to a diagnosis at or greater than S3 among our set of individuals, we may expect more than 200 individuals (30.9% of the sample) to be at considerable risk of TT development within this study alone. A systematic review by Cromwell et al[43] also demonstrated the persistence of disease burden among women beyond scarring, as 17 of 24 included studies demonstrate a significant difference in odds of trichiasis among women versus men. Nine of thirteen (69.2%) observed trichiasis cases within the current sample were among women, demonstrating an unequal burden of TT expected within this population consistent with previous findings.

Limitations Several limitations are noted within this study. While primary graders were competent and certified in utilizing the 4-point grading scale proposed by Wolle et al,[26] they were not trained to discriminate clinical signs due to other stages of trachoma development (e.g. presence of follicles or severe inflammation). Clinical signs of follicular and inflammation stages were often in co-occurrence with scarring due to the endemicity status of the region and prevented full visualization of any blood vessel obstruction, which may contribute to the considerable number of ungradable photographs deemed at the eye-level. One other potential limitation of our study is that a significant proportion (15.6%) of the analyzed photograph were classified as grade S1, which are reportedly challenging to grade accurately by Cox et al.[23] This limitation was addressed by the use of multiple graders to reach a person-level consensus and ensure specificity and sensitivity of the disease screening. However, nearly a quarter of the photograph pairs (i.e., eye-level) recorded as S1 required adjudication. Concerning primary outcomes, we recognize that household surveillance for trachoma diagnosis tends to underrepresent the male population.[43] However, the response rate was comparable to that of previous trachoma impact surveys in the region and other hyperendemic communities surveyed by other programs.[51,52] Additionally, the prevalence of TF was used as a proxy for ongoing *C. trachomatis* transmission which may not always be accurate, as TF is a lagging indicator that takes a long time to disappear even after *C. trachomatis* had disappeared.[9] Other reports of this photographic sample have noted a favorable and representative response rate for in-field household surveys and photograph collection.[30] Very few adults with in-field examinations were not photographed or had less than two photos per eye collected. With one photographer experienced in trachoma studies for the duration of the 2017 trachoma impact survey,

photographs were collected alongside one of four in-field examination teams on any given day. Thus, not all clusters were surveyed using photography. Given the randomization and geographical distribution of clusters selected for photographic grading, we expect that the photographic grading sample remains a population-based sample of ten communities within the Amhara region.

Recommendations This study supports the pursuit of scarring severity surveillance systems within Amhara and other hyperendemic regions, to characterize the portion of the population at risk for trichiasis development. So far, the limited understanding of the scarring pathogenesis has resulted in the absence of a treatment or recommendation capable of halting progression. Observational data from scarring surveillance, particularly surrounding the distribution of scarring among subpopulations, could improve modeling techniques used to predict incident TT to come and better inform implementation strategies for trichiasis surgery programs. We provided evidence of the excessive burden that women experience in endemic communities, which suggests that programs should focus uptake of SAFE and particularly TT surgery efforts on this vulnerable population as communities strive towards elimination. It is important to note that targeted surveillance may not only benefit women. Our study has reinforced that there is a level of clustering within communities that may be susceptible to trichiasis development. It is essential for trachoma control programs, particularly those formerly-hyperendemic regions, to establish surveillance systems that can effectively identify, target, and treat incident cases of TT within vulnerable subpopulations after elimination thresholds have been reached.

Conclusions

This study revealed that several individual and household factors, including being female, older age, and living in a district where the prevalence of TF is higher than 10% are independent factors that are associated with increasing scarring severity within this hyperendemic community. This suggests that a significant portion of the population may be susceptible to scarring progression and trichiasis development, with the potential to exacerbate disparities amongst subpopulations. This study highlights the significant threat that trachoma elimination faces, emphasizing the need for continuous surveillance of scarring trends in hyperendemic communities to prepare for future challenges.

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Appendix

Figure 1. Age- specific distribution of trachomatous scarring severity among adults (> 15 years), Amhara, Ethiopia.

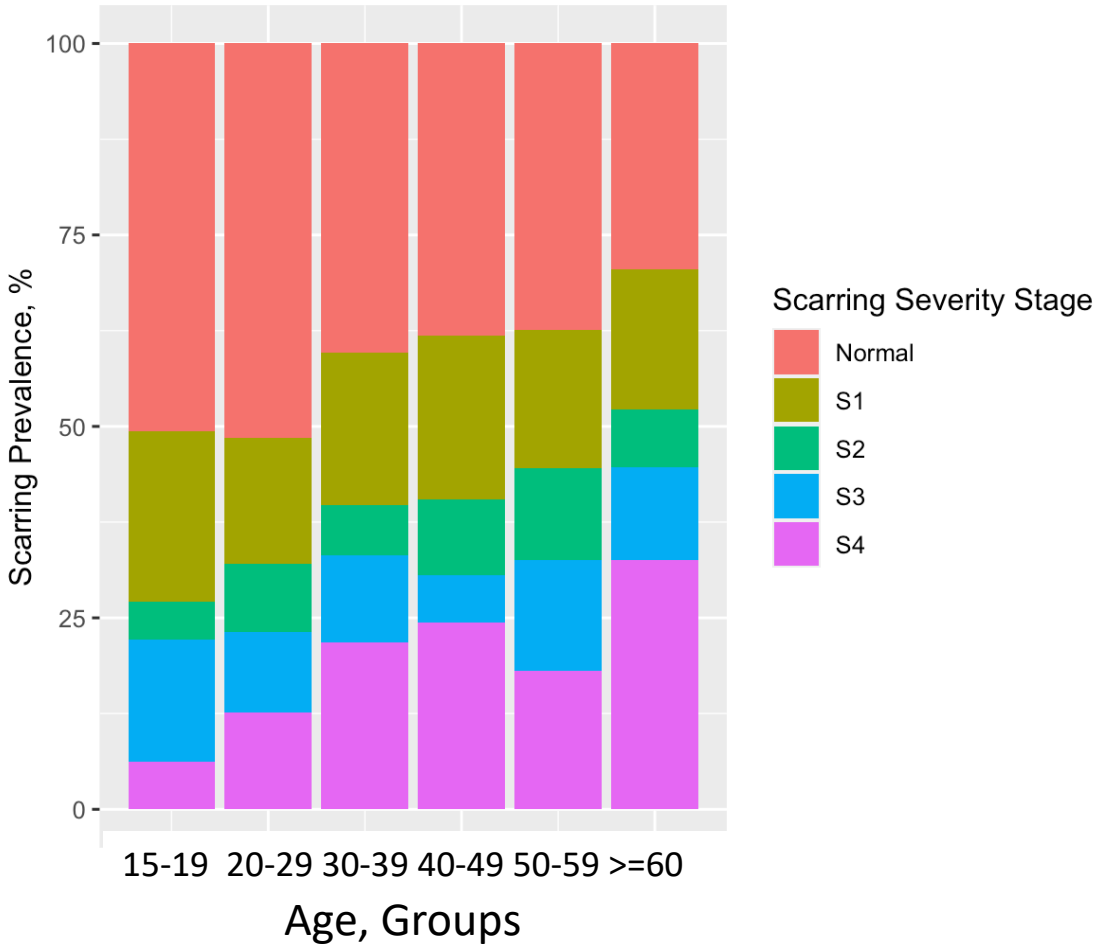


Figure 2. Gender- specific distribution of trachomatous scarring severity among adults (> 15 years), Amhara, Ethiopia.

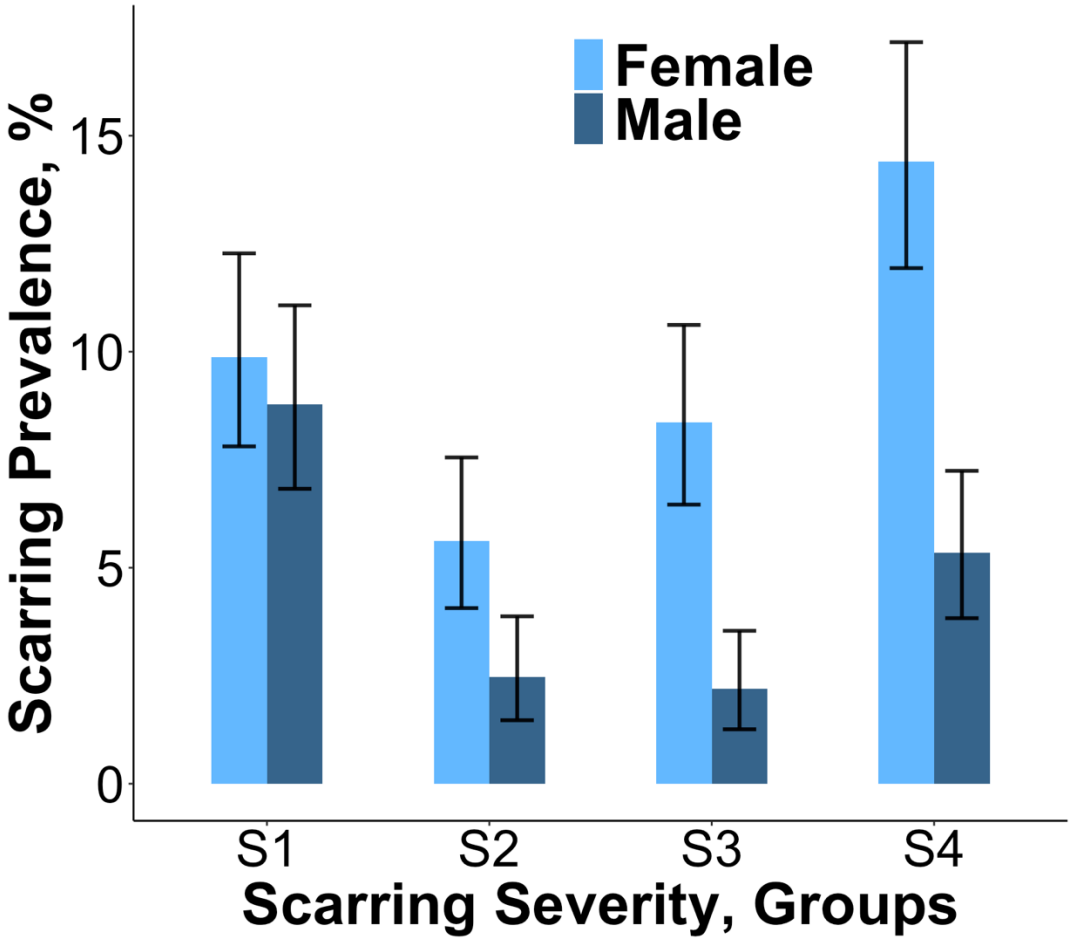


Table 2. Characteristics of 2017 Participants in Photographic Study)

	Overall	Scarring Absent	S1	S2	S3	S4
N (%)	729	291 (39.9)	137 (18.8)	59 (8.1)	80 (11.0)	145 (19.9)
Age, mean ± SD	41.5 ± 18.3	37.7 ± 16.6	40.8 ± 18.9	42.6 ± 18.2	40.9 ± 19.5	48.0 ±18.6
Sex (%) [a]						
Male	266 (36.7)	123 (42.3)	64 (46.7)	18 (30.5)	16 (20.0)	39 (27.1)
Female	458 (63.3)	168 (57.7)	72 (52.5)	41 (69.5)	61 (76.3)	105 (72.9)
WASH Characteristics						
Lack of Improved Water (%)	185 (25.4)	59 (31.9)	29 (15.7)	17 (9.2)	32 (17.3)	43 (23.2)
Latrine Absent (%)	467 (64.1)	172 (36.8)	77 (16.5)	42 (9.0)	61 (13.1)	104 (22.3)
CO or TT (%)	31 (4.3)	1 (3.2)	0 (0.0)	2 (6.5)	2 (6.5)	25 (80.6)

[A] Missing Sex(N)= 5

Table 3. Individual factors for Trachomatous Scarring Severity

Categorical Variable	OR	95% Confidence Interval
Age		
15-19	1.00	--
20-30	1.08	0.65-1.80
30-40	1.72	1.05,2.83
40-50	1.82	1.10, 3.04
50-60	1.82	1.05, 3.17
60-74	2.36	1.35, 4.13
75+	4.24	2.20, 8.28
Sex		
Male	1.00	--
Female	1.73	1.31-2.30

Table 4. Household and District factors for Trachomatous Scarring Severity

Categorical Variable	OR	95% Confidence Interval
Latrine Use		
Yes	0.68	0.51-0.90
No	1.00	--
Improved Water Source		
Yes	0.62	0.45-0.84
No	1.00	--
TF Prevalence		
<10%	1.00	--
>=10%	4.33	3.23, 5.83

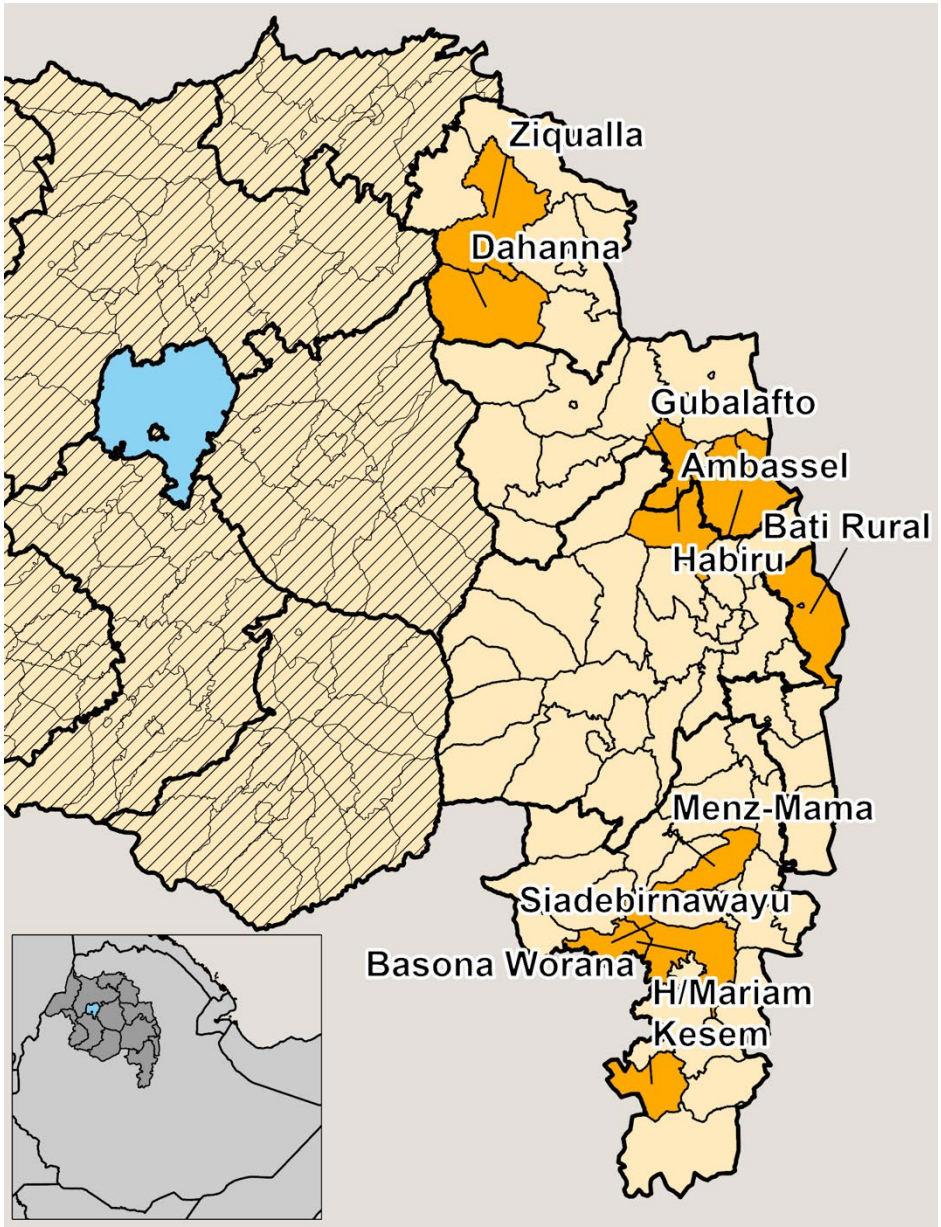
Table 5. Adjusted random effects logistic model for trachomatous scarring severity (n=707)

Categorical Variable	OR	95% Confidence Interval
Age		
15-19	1.00	--
20-30	1.50	0.84, 2.67
30-40	1.95	1.10, 3.42
40-50	2.24	1.27, 3.95
50-60	3.00	1.58, 5.60
60-74	4.57	2.44, 8.48
75+	10.00	4.71, 27.26
Sex		
Male	1.00	--

Female	1.77	1.28, 2.44
TF Prevalence		
<10%	1.00	--
>=10%	6.03	1.33, 27.26

Supplemental Appendix

Supplemental Figure 1. Surveyed Districts in East Amhara, Ethiopia (2017 Trachoma Impact Survey)



Supplemental Figure 2. Distribution of trachomatous scarring and severity among adults (> 15 years), Amhara, Ethiopia