Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India

M Srinivasan, Christine A Gonzales, Celine George, Vicky Cevallos, Jeena M Mascarenhas, B Asokan, John Wilkins, Gilbert Smolin, John P Whitcher

Abstract

Aims/background—To determine the epidemiological characteristics and risk factors predisposing to corneal ulceration in Madurai, south India, and to identify the specific pathogenic organisms responsible for infection.

Methods—All patients with suspected infection central corneal ulceration presenting to the ocular microbiology and cornea service at Aravind Eye Hospital, Madurai, from 1 January to 31 March 1994 were evaluated. Sociodemographic data and information pertaining to risk factors were recorded, all patients were examined, and corneal cultures and scrapings were performed.

Results—In the 3 month period 434 patients with central corneal ulceration were evaluated. A history of previous corneal injury was present in 284 patients (65.4%). Cornea cultures were positive in 297 patients (68.4%). Of those individuals with positive cultures 140 (47.1%) had pure bacterial infections, 139 (46.8%) had pure fungal infections, 15 (5.1%) had mixed bacteria and fungi, and three (1.0%) grew pure cultures of Acanthamoeba. The most common bacterial pathogen isolated was Streptococcus pneumoniae, representing 44.3% of all positive bacterial cultures, followed by Pseudomonas spp (14.4%). The most common fungal pathogen isolated was Fusarium spp, representing 47.1% of all positive fungal cultures, followed by Aspergillus spp (16.1%).

Conclusions—Central corneal ulceration is a common problem in south India and most often occurs after a superficial corneal injury with organic material. Bacterial and fungal infections occur in equal numbers with Streptococcus pneumoniae accounting for the majority of bacterial ulcers and Fusarium spp responsible for most of the fungal infections. These findings have important public health implications for the treatment and prevention of corneal ulceration in the developing world.

Central corneal ulceration is a major cause of monocular blindness in developing countries. Surveys in Africa and Asia have confirmed this finding, and a recent report on the causes of blindness worldwide consistently lists corneal scarring second only to cataract as the major aetiology of blindness and visual disability in many of the developing nations in Asia, Africa, and the Middle East.

Considering the importance of ulceration as a worldwide cause of monocular visual loss, there are surprisingly few studies evaluating the aetiological factors predisposing a population to corneal infection. Several investigators have reported the prevalence of bacterial and fungal pathogens isolated from ulcerated corneas, but until now there has not been a population based study demonstrating the true incidence of microbial keratitis in a developing country. Gonzales et al have recently completed such a study which documents the incidence of corneal infection in Madurai District, south India. The annual incidence was conservatively estimated to be 11.3 per 10 000 population or at least 10 times more frequent than the incidence of similar infections in an age and sex adjusted population in the USA. Extrapolating from this estimated annual incidence, over a 10 year period 1% of the entire population of Madurai District, or over 35 000 individuals, will develop a central corneal ulcer. Thus, ulceration of the cornea in south India is a blinding disease of epidemic proportions.

Of significance is a preliminary report from the same area that 44% of all central corneal ulcers are caused by fungi. This high prevalence of fungal pathogens in south India is significantly greater than that found in similar studies in Nepal (17%), Bangladesh (36%), and south Florida (35%). The severity of these infections and the poor response to treatment of most fungal ulcers means that these eyes are invariably blinded or lost.

The epidemiological pattern of corneal ulceration varies significantly from country to country and even from region to region. In order to develop a comprehensive strategy for the diagnosis, treatment, and ultimately for the prevention of corneal infections, the aetiological factors predisposing to ulceration and the pathogenic organisms which are responsible must be determined. Answers to these questions are crucial if a programme of prevention is to be considered and if appropriate therapeutics measures are to be instituted.

The purpose of this study was to evaluate all of the infectious central corneal ulcers seen at Aravind Eye Hospital in Madurai, south India, over a 3 month period from 1 January to 31 March 1994. We documented the antecedent..
factors predisposing to the onset of corneal ulceration and identified the specific organisms responsible for infection.

Methods

Patients

All patients with infectious central corneal ulcers presenting to the oculomicrobiology and cornea service at Aravind Eye Hospital, Madurai, south India from 1 January to 31 March 1994 were included in the study. Aravind Eye Hospital is a large referral centre and postgraduate training institute that provides eye care for patients from all over south India. Patients were seen consecutively after the initial clinical diagnosis of corneal ulceration was made. Ulceration was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon. Typical viral ulcers and healing ulcers were excluded as were Moore’s ulcers, marginal ulcers, interstitial keratitis, sterile neurotrophic ulcers, and any ulcers associated with autoimmune conditions. A standardised form was filled out on each patient documenting sociodemographic information as well as clinical information including duration of symptoms, previous treatment, predisposing ocular conditions, and associated risk factors.

Clinical Procedures

Every patient was examined at the biomicroscope by an ophthalmologist. The size of the epithelial defect after staining with fluorescein was measured with the variable slit on the biomicroscope and recorded in millimetres on a standardised form. In similar fashion the size and depth of the stromal infiltrate was recorded. A sketch of each ulcer was also drawn on the form using standardised frontal and cross sectional diagrams, and the presence or absence of a hypopyon was recorded and the height measured in millimetres. Associated ocular conditions such as blepharitis, dacryocystitis, dry eyes, corneal anaesthesia, or ocular leprosy were noted.

After a detailed ocular examination corneal scrapings were performed under aseptic conditions on each ulcer by an ophthalmologist using a flame sterilised Kimura spatula. Scrapings were performed at an operating microscope after instillation of 4% lignocaine (lidocaine) without preservatives. Material obtained from scraping the leading edge and the base of each ulcer was inoculated directly onto sheep’s blood agar, chocolate agar, and dextrose agar (PDA), and into brain heart infusion broth (BHI) without gentamicin sulphate. Material from the corneal scraping was also smeared on three separate glass slides: one for Gram stain, one for Giemsa stain, and the third for microscopic examination in the clinic as a KOH wet mount. All KOH smears were then sent to the laboratory for confirmation. When KOH smears were positive for amoebic cysts a further corneal scraping was performed and the material was inoculated onto non-nutrient agar overlaid with Escherichia coli in an attempt to isolate Acanthamoeba spp.

Laboratory Procedures

All bacterial cultures were incubated aerobically at 37°C. Cultures on blood agar and chocolate agar were evaluated at 24 hours and at 48 hours and then discarded if there was no growth. Cultures inoculated in BHI were examined in similar fashion. Fungal cultures inoculated onto PDA were incubated at 27°C, examined daily, and discarded at 1 week if no growth was present. Cultures on non-nutrient agar overlaid with E coli were examined daily for the presence of Acanthamoeba spp and likewise discarded at 1 week if there were no signs of growth. All laboratory methods followed standard protocols which have been discussed in detail in a previous report.

Microbial cultures were considered positive only if growth of the same organism was demonstrated on two or more solid media; or there was semiconfluent growth at the site of inoculation on one solid medium associated with the identification of the organism of appropriate morphology and staining characteristics on Gram or Giemsa stained corneal smears. Cultures for Staphylococcus epidermidis and diphtheroids were considered positive only if there was moderate growth on at least two solid media. Liquid media were found to be so easily contaminated that they could not be relied upon for accurately identifying organisms. The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria. Fungi were identified by their colony characteristics on PDA and by their microscopic appearance in lactophenol cotton blue.

Results

Epidemiological Characteristics

From 1 January to 31 March 1994 there were 434 patients with the clinical diagnosis of central corneal ulceration that were examined at the cornea service at Aravind Eye Hospital. All 434 met the criteria for the clinical diagnosis of...
central corneal epithelial defect with underlying stromal infiltration and suppuration with signs of inflammation. In addition, at the initial examination 232 patients (53.5%) had a documented hypopyon. Of the total 434 patients 266 (61.3%) were males and 168 (38.7%) were females with ulceration occurring in both groups most frequently in the middle decades of life (Fig 1). The predominance of corneal ulceration in males was most pronounced in the middle years with an overall ratio of male to female patients of 1.6 to 1.

The occupations of the patients reflected a cross section of the work force in Madurai City and the surrounding agricultural area (Table 1). The majority were farmers or hired agricultural workers, usually working in village rice or sugar cane fields. The duration of the patients’ symptoms before their evaluation at Aravind Eye Hospital was also determined (Table 2). Most of the patients were examined within 7 days after the onset of their illness. The greatest number (182 patients, 41.9%) were seen from day 4 to 7, but 26 patients waited for more than a month before coming to the hospital for evaluation.

Medical help was sought by 310 (71.4%) of the 434 patients before their initial examination at Aravind Eye Hospital. Of the 310 who received medical attention 203 patients (65.4%) were seen by eye physicians, 34 (11.0%) went to general physicians, 27 (8.7%) received advice from chemists or pharmacists, and five (1.6%) went to homeopaths or health assistants. It is of interest that 62 patients (20.0%) went to village healers. In several instances patients consulted both physicians and village healers before presenting for examination.

Medical treatment was started on 376 (86.6%) of the 434 patients before coming to Aravind Eye Hospital. In many cases the patients purchased medication without benefit of an examination or after a brief visit to a physician or village healer.

Of the 376 patients who presented on some form of topical medication, 217 (57.7%) were taking topical antibiotics, usually ciprofloxacin, gentamicin, chloramphenicol, or sodium sulphacetamide; 37 patients (9.8%) were taking antifungal drops, usually natamycin; 30 patients (8.0%) were taking topical corticosteroids; and 23 (6.1%) were taking unknown drops. Topical antibiotics, antifungals, and corticosteroids were used either alone or in combination, usually depending on the severity of the ulcer. Fifty eight (13.4%) of the 434 patients were taking no drops of any kind at the time of examination.

It is of interest that 162 (37.3%) of the total of 434 patients were using or had recently used some kind of herbal topical medication before examination. These traditional remedies were frequently used associated with or instead of antibiotic and antifungal medications. Of the 162 patients, 42 (25.9%) had put breast milk into the eye, a total of 9.7% of all 434 patients. Thirty two (19.8%) of the 162 patients using herbal medicine had put some kind of oil into the eye (castor oil, lamp oil, or seed oil) and nine patients (5.6%) had applied plant juice (usually from a flower or onion extract). One patient put honey in her eye, one used steam to cleanse the cornea, and three dropped chicken blood into the eye. It is also of interest that in six patients with corneal foreign bodies before ulceration, an attempt was made by a relative or a village healer to remove the foreign body with the tip of the tongue.

**RISK FACTORS**

A history of recent injury to the cornea was obtained in 284 (65.4%) of the 434 patients. Agents responsible for the trauma were mainly agricultural and animal products (Table 3). Other complicating conditions predisposing to corneal ulceration were present in 65 (15.0%) of the 434 patients. These conditions included chronic dacryocystitis in 19 patients and a mucocele in one patient, leprosy in eight patients, non-leprous lagophthalmos in seven patients, Bell’s palsy with exposure keratitis in two patients, ectropion in one patient, coma in one patient, severe blepharitis in two patients, and keratoconjunctivitis sicca in one patient.

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**Table 1** Occupations of 434 patients with corneal ulceration in south India

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural worker/farmer</td>
<td>245</td>
<td>56.4</td>
</tr>
<tr>
<td>Housewife/domestic</td>
<td>52</td>
<td>12.0</td>
</tr>
<tr>
<td>Labourer*</td>
<td>45</td>
<td>10.4</td>
</tr>
<tr>
<td>Tradesman/profession†</td>
<td>35</td>
<td>8.1</td>
</tr>
<tr>
<td>Student/child</td>
<td>29</td>
<td>6.7</td>
</tr>
<tr>
<td>Unemployed/unknown</td>
<td>28</td>
<td>6.4</td>
</tr>
<tr>
<td>Total</td>
<td>434</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*R An individual, either male or female, who does heavy manual labour, lifting, loading, and carrying of materials usually balanced on the head.
† Middle class workers such as mechanics, stone masons, electricians, carpenters, pipe fitters, welders, and fishermen. This category also includes professions such as teachers, police, office workers, factory workers, drivers, and merchants.

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**Table 2** Duration of symptoms in 434 patients with corneal ulceration in Madurai, south India

<table>
<thead>
<tr>
<th>Duration of symptoms</th>
<th>No of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 days</td>
<td>82</td>
<td>18.9</td>
</tr>
<tr>
<td>4–7 days</td>
<td>182</td>
<td>41.9</td>
</tr>
<tr>
<td>8–14 days</td>
<td>81</td>
<td>18.7</td>
</tr>
<tr>
<td>15–29 days</td>
<td>58</td>
<td>13.4</td>
</tr>
<tr>
<td>1–2 months</td>
<td>22</td>
<td>5.1</td>
</tr>
<tr>
<td>&gt;2 months</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>Unspecified</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td>434</td>
<td>100.0</td>
</tr>
</tbody>
</table>

---

**Table 3** Traumatic agents in 284 (65.4%) of 434 patients with corneal ulceration

<table>
<thead>
<tr>
<th>Traumatic agent</th>
<th>No of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddy</td>
<td>72</td>
<td>25.4</td>
</tr>
<tr>
<td>Tree branch or thorn</td>
<td>53</td>
<td>18.7</td>
</tr>
<tr>
<td>Dust, soil, or stones</td>
<td>51</td>
<td>18.0</td>
</tr>
<tr>
<td>Vegetable matter*</td>
<td>45</td>
<td>15.1</td>
</tr>
<tr>
<td>Animal matter†</td>
<td>16</td>
<td>5.6</td>
</tr>
<tr>
<td>Metallic foreign body</td>
<td>14</td>
<td>4.9</td>
</tr>
<tr>
<td>Miscellaneous object‡</td>
<td>14</td>
<td>4.9</td>
</tr>
<tr>
<td>Finger nail</td>
<td>12</td>
<td>4.2</td>
</tr>
<tr>
<td>Unknown</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>Total</td>
<td>284</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Organic matter, hay, sugar cane, grass, corn stalks, wood, onions, groundnuts, leaf.
† Cow’s tail, cow dung, insect.
‡ Metal objects, chemicals, paint, fish nets, broom sticks, cricket balls, cloth, etc.
Eight patients had corneal anaesthesia following herpes simplex or herpes zoster infections, and one patient had neurotrophic keratitis for other reasons. In addition, four patients were diabetic, nine patients had climatic droplet keratopathy, and one had band keratopathy. In contrast with the patients who had a definite history of corneal trauma, the risks for corneal ulceration associated with these conditions were presumptive.

**MICROBIOLOGICAL DIAGNOSIS**

Cultures were positive and fulfilled the criteria established for the presence of infection in 297 (68.4%) of the 434 corneal ulcers (Table 4). Pure bacterial growth was present in 167 isolates, 140 (47.1%) had pure bacterial infections, 139 (46.8%) had pure fungal infections, 15 (5.1%) had mixed bacterial and fungal infections, and three (1.0%) had pure Acanthamoeba infections.

A total of 167 bacterial organisms were cultured from 155 corneal ulcers (Table 5). Of the 167 isolates, 132 (79.0%) were Gram positive and 35 (21.0%) were Gram negative. *Streptococcus pneumoniae* was the most commonly isolated bacterial organism representing 74 (44.3%) of all positive bacterial cultures. The next most commonly isolated Gram positive organism was *Corynebacterium xerosis* with 21 (12.5%) positive cultures. Of these 21 cultures, seven were pure, six were mixed with other bacteria, and eight mixed with fungi. *Staphylococcus epidermis* was cultured from 17 patients (10.2%) and *Staphylococcus aureus* from nine patients (5.4%). *Streptococcus viridans* and *Bacillus* spp occurred rarely. Seven cultures positive for *Nocardia asteroides* were also included under Gram positive bacterial species. *Pseudomonas* spp was isolated from 24 cultures (14.4%) and was the most frequently occurring Gram negative organism. Other Gram negative organisms infrequently cultured included *Moraxella* spp, *Haemophilus influenzae*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Aeromonas hydrophila*, and *Escherichia coli*. Of the 167 positive bacterial cultures from corneal ulcer patients, 128 cultures (76.6%) were pure isolates, 24 (14.4%) were mixed with another species of bacteria, and 15 (9.0%) were mixed with a single fungal species.

A total of 155 fungal organisms were cultured from 154 corneal ulcers (Table 6). Of 155 total isolates, 73 were *Fusarium* spp (47.1%), 25 were *Aspergillus* spp (16.1%), and six were *Botryodiplodia theobromae* (3.9%). Other fungal species were cultured in decreasing frequency as well as 19 unidentified dematiaceous fungal species and 14 unidentified hyaline fungal species. Dematiaceous fungi are pigmented filamentous organisms such as *Curvularia* and *Cladosporium*. Hyaline fungi, or moniliaceae, are non-pigmented filamentous organisms such as *Fusarium* and *Aspergillus*. Non-filamentous fungal pathogens were isolated from any of the patients. Of all the fungal organisms cultured from patients with ulcer, 138 cultures (89.0%) were pure isolates, two (1.3%) were mixed with other fungal species, and 15 (9.9%) were mixed with a corresponding single species of bacteria.

Three patients had positive cultures for *Acanthamoeba*. Only one of the three gave a history of earlier trauma to the eye. None of the total of 434 patients in the study wore contact lenses.

**Discussion**

Undoubtedly the prevalence of visual disability from corneal opacity varies from one geographical location to another, and in areas in the world where trachoma, onchocerciasis, leprosy and other infectious causes of eye disease are endemic, blindness rates may be as high as...
Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India

969

...3% of the entire population. In south India reliable statistics documenting the prevalence of blindness from corneal scarring are not available, but the incidence of corneal ulceration is more than 10 times higher (11.3 per 10 000) than in a comparable population in the USA, and in Madurai District alone an estimated 3500 individuals are affected each year. Males in their sixth decade of life are particularly at risk. Overall, the ratio of male to female patients with corneal ulceration is 1.6 to 1, the same ratio reported by Gonzales et al. Both sexes tend to develop corneal ulcers in the middle decades of life when presumably they are more physically active and at a higher risk for corneal injury. The majority of ulcer patients were agricultural workers, home makers, or labourers (78.8%), an occupation profile similar to Nepal (72%) but in marked contrast with Ghana where only 16.1% of the patients were involved in agricultural activity. Most of the south Indian patients were engaged in heavy labour, either in an agricultural setting, in construction, or in physical transport of heavy materials.

It is of interest that over 60% of the patients in the study presented for examination during the first week of their illness. Transportation is excellent in southern India and patients travel great distances by train and bus to Madurai to be seen at Aravind Eye Hospital because of its reputation for excellent eye care. The relatively short duration of symptoms before examination is an interesting contrast with findings in Nepal where only 43.7% of patients were seen in the first week of their illness and 19.3% took longer than 1 month to make the journey to the hospital. The majority of patients in south India appear to have access, even if somewhat limited, to relatively sophisticated eye care. Before their initial examination 71.4% of all cultures were positive, but it does not approach the 80% isolation rate reported from Nepal by Upadhyay et al or the recovery rate of 81.7% reported by Dunlop et al in Bangladesh. Even though multiple scrapings from the area of ulceration were performed and enriched media were used for inoculation as in the Nepal study, where there was an 80% recovery rate, tell lower rate of isolation was attributed to the more widespread availability of topical medications in south India and their use in the majority of patients (86.6%) before evaluation.

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Almost half (46.8%) of all corneal ulcers with positive cultures were fungal in origin. If the 15 mixed infections (5.1%) are also considered to be primarily fungal for treatment purposes and they are added to the pure fungal cases, 51.9% of all culture positive corneal ulcers grew fungal pathogens. This figure approaches the fungal isolation rate in Ghana where Hagan et al. cultured fungi from 56% of 114 patients who had positive cultures. Of the 167 bacterial isolates from 155 corneal ulcers, 44.3% were Streptococcus pneumoniae. This number is similar to findings in Nepal where Streptococcus pneumoniae was again the most frequently isolated bacterial pathogen (31.1%). Carmichael et al. also reported Streptococcus pneumoniae as the most common cause of bacterial corneal ulceration (39.6%) in a similar group of African patients who were either manual labourers or unemployed. Unlike the industrialised world, where Pseudomonas spp and other opportunistic organisms are the most frequent causes of bacterial ulceration because of contact lenses and other risk factors, in the developing world Streptococcus pneumoniae should always be considered as the most likely cause of bacterial corneal ulceration until proved otherwise. Even though Pseudomonas spp has been identified as a frequent cause of corneal ulceration in some developing countries, the importance of Streptococcus pneumoniae as a worldwide cause of corneal infections should not be underestimated because of the implicit need for adequate therapy with broad spectrum antibiotics for all ulcers until an aetiological diagnosis has been made. Other important causes of bacterial ulceration included Staphylococcus spp (15.6%), Pseudomonas spp (14.4%), and Corynebacterium xerosis, or diphtheroids (12.5%). One third of diphtheroids were found in pure culture while the remainder were mixed evenly between bacterial and fungal infections. Diphtheroids are undoubtedly a primary cause of corneal ulceration as demonstrated in a report of eight cases by Rubinfeld et al. In Nepal Upadhyay et al. reported 11 ulcers from which only diphtheroids were cultured.

Of 155 fungal isolates cultured from 154 corneal ulcers 47.1% were Fusarium spp, 16.1% were Aspergillus spp, and the remaining organisms were a diverse mixture of unusual fungal pathogens including a large number of unidentified dematiaceous fungal species (13.3%) and hyaline fungal species (9.6%). The fact that many of these organisms could not be adequately speculated and that in some cases the genus was also difficult to determine has been reported by other investigators. Various species of fungi can be easily described by a single spore culture. In this study any filamentous organisms that could not be positively identified by standard laboratory methods were listed as unidentified pigmented (dematiaceous) or non-pigmented (hyaline) filamentous fungi. No non-filamentous fungi were cultured from any of the patients. This pattern of fungal organisms, dominated by Fusarium spp, is similar to the spectrum of microbial keratitis reported from south Florida by Liesegang and Forster and from Ghana by Hagan et al. In south Florida 35% of the isolated organisms were fungi with Fusarium spp accounting for 61%. In Ghana fungi alone or in combination with bacteria were isolated from 56% of all culture positive patients. Fusarium spp accounted for 52% of all fungal isolates. The climates of south Florida and Ghana are similar in many respects to the climate of south India, which may explain the corresponding pattern of fungal organisms. By contrast, in most of the world Aspergillus spp or Candida spp are the predominant fungal pathogens responsible for mycotic keratitis. In the temperate climate of Nepal Upadhyay et al. found that Aspergillus spp accounted for 47% of all fungal pathogens followed by Candida spp (13.2%) and Fusarium spp (11.7%). Obviously, fungal keratitis is an enormous public health problem in south India. The fact that half of all corneal ulcers seen at Aravind Eye Hospital were fungal in origin and that the majority were caused by Fusarium spp, one of the most virulent ocular pathogens, underscores the need for more effective methods of diagnosis and treatment to decrease the burden of avoidable blindness.

This study was developed primarily to determine the specific pathogens responsible for corneal ulceration in south India. An attempt was also made to identify the epidemiological characteristics of the population at risk for corneal ulceration as well as those factors which predispose to the development of an ulcer. As in our previous study in Nepal, we feel that comprehensive surveys such as this are necessary to assess the specific epidemiological characteristics of corneal ulceration which are unique for each region and population. This knowledge is essential, firstly, to define the magnitude of the problem in terms of health care costs, human costs, and the economic burden of blindness and, secondly, to design an efficient public health programme for the rapid referral, diagnosis, treatment, and ultimately the prevention of corneal ulceration in the population at risk. This approach has important public health implications for the treatment and prevention of corneal ulceration in the developing world.

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