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CORNEA - I

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Acinetobacter Baumannii Keratitis: A Great Masquerade-16 Case Series at Tertiary Eye Care Centre

Dr. Milap Vaghela, Dr. Murthy Somasheila I, Dr. Savitri Sharma

Acinetobacter Baumannii is a pleomorphic, Gram negative, aerobic bacterium which is generally an inhabitant of soil, vegetative material and aquatic environment. It is an opportunistic pathogen and transmitted by healthcare providers. This organism is well known since decades for its propensity to cause serious life threatening hospital acquired infections in critically ill patients like respiratory tract infections, meningitis, necrotising fasciitis, septicaemia etc. Hospital acquired pneumonia is the most common infection caused by this organism. The major difficulty while tackling with these infections is existence of evolved multidrug resistant (MDR) species of A. Baumannii which causes a significant burden on healthcare and established themselves as “red alert” human pathogen.

However, A. Baumannii is a rare cause of ocular infection and very few case reports are available in literature on A. Baumannii keratitis. There are reports in the literature of exposure keratitis, corneal ulcer after penetrating keratoplasty, injury induced endophthalmitis, endophthalmitis and surgical site infection (SSI) following cataract surgery, corneal perforation, infectious crystalline keratopathy and corneal infection in soft contact lens wearers.

The answer to the question why it is important to study these organisms lies in their ability to increase the chances of aggressive ocular infection after mere colonization of ocular surface due to their transferable MDR nature and their ability to resist eradication by pre operative antibiotic therapy.

Our case series is the largest till date describing varied clinical presentation of A. Baumannii keratitis and challenges in diagnosing this condition as it is often initially misdiagnosed in majority of cases.

MATERIALS AND METHODS

This is a retrospective descriptive case series. Approval of the Institutional review board was obtained. All cases of culture positive A Baumannii keratitis from 2005 to 2013 were identified from the microbiological
archives and the medical records of these cases were reviewed and data was collected with the following inclusion criteria.

**Inclusion criteria**
- Culture positive Acinetobacter Baumannii keratitis in all age groups

**Exclusion criteria**
- Culture negative cases
- Culture positive cases which were lost to follow up

All cases underwent comprehensive ocular examination followed by corneal scraping; except in 2 cases which presented with corneal perforation. One of these underwent TABCL and the other underwent patch graft. Samples of corneal exudates were taken during the surgical intervention. As per our standard institute protocol, all samples were sent for Gram, KOH, Calcoflour white and Giemsa smears. For cultures, inoculation was done in Blood agar, Chocolate agar, Sabouraud Dextrose Agar, Potato Dextrose Agar, BHI agar, nutrient broth and Thioglycolate broth. Samples also sent for PCR. Cultures were observed for minimum period of 7 days for growth.

Initial treatment in all cases was started on the basis of smear report/clinical picture. Treatment revised subsequently on basis of culture reports and modified afterwards as per clinical response on follow up visits.

**RESULTS**
A total of 16 patients were found having culture positive Acinetobacter baumannii keratitis, out of which 68.75% were male and 31.25% were female. Right eye was involved in 50% and left eye was involved in other 50% patient. Majority of the patients were found between the age group of 31 to 50 yrs with average age of presentation was 50.12 years. (Figure 1). Mean duration of follow up was 173.4 days.

On slit lamp biomicroscopy , the clinical picture varied widely. One subgroup of patients presented with rapid onset of symptoms without

![Figure 1: Many patients were having ocular predisposing factors / compromised cornea. (Figure 2). The presenting visual acuity in majority of patients were HM+ (hand movements+).](image1)

![Figure 2: s/p PK-status post penetrating keratoplasty](image2)
antecedent trauma having epithelial defect (black arrow) with stromal infiltrate with hypopyon (orange arrow) without satellite lesions or immune ring resembling bacterial keratitis as depicted in (Figure 4). Provisional diagnosis of microbial keratitis presumed to be bacterial origin was made.

Second subgroup of patients presented with stromal interstitial keratitis (Figure 5) with stromal affection without epithelial defect or necrotic keratitis (Figure 6) like picture which clinically resemble viral keratitis. On clinical assumption, topical/systemic antivirals were started but patients were not benefitted.

Third subgroup of patients presented with dry looking ulcer with hypopyon with satellite lesions resembling fungal keratitis. However, history of trauma with vegetative material was absent (Figure 8).

One of the severe clinical presentation was with corneal perforation which required immediate surgical treatment.
The smear reports correlated only in 4 of 16 patients. Four showed Gram negative bacilli [GNB]. Smear reports were as depicted in Table 1.

The clinical features therefore had a wide range of spectrum as depicted in Figure 9. All patients were initially started on intensive antibiotics (fortified cefazolin 2.5 % and ciprofolxacin 0.3% eye drops). Treatment was modified according to antibiotic sensitivity report.

Antibiotic sensitivity showed more than 93% of the isolates were sensitive to fluoroquinoloes, with gatifloxacin appearing to be having least drug resistance. (Figure 10)

Out of 16 patients, 31.25% required Therapeutic PK. Outcome of PK is shown in Figure 11.
DISCUSSION

A. Baumannii keratitis is a rare ocular infection, but may poses a major challenge in initial difficulty in diagnosis, both clinically and on microbiology, as well as treatment.

Acinetobacters may be identified presumptively to the genus level as gram-negative, catalase-positive, oxidase-negative, nonmotile, nonfermenting coccobacilli. They are short, plump Gram negative rods that are difficult to destain and can be misidentified as either Gram negative or Gram positive cocci. On routine solid culture media (e.g. sheep blood agar), A.calcoaceticus- A. Baumannii complex produce smooth, mucoid, greyish white colony having diameter of 1.5 to 3 mm after overnight culture which on morphology resembles Enterobacteriaceae. Smears of A. Baumannii sometimes also show Gram negative intracellular diplococci resembling Nisseria. Since most Acinetobacter isolates are resistant to penicillin and chloramphenicol, while members of the genus Neisseria are sensitive to these drugs, the ophthalmologist must be aware of the similarity between these two genera in smears.

In our study, we also found that smear results were misleading in the initial stage of diagnosis. Only 4 out of 16 have shown GNB. Rest showed GPC, fungus and no organisms.

There are multiple factors which contribute to pathogenecity and MDR potential of A. Baumannii. Ocular isolates of A. Baumannii are clonally distinct. A. Baumannii possesses multiple plasmids with transferable antibiotic resistant traits to other bacteria and causes dissemination of antibiotic resistance among ocular flora. They produce biofilm surrounding them making their infection difficult to eradicate. They have capability to adhere and invade corneal epithelium and thereby producing cellular toxicity and cell death.

Case reports have also shown that A. Baumannii keratitis / endophthalmitis also occurs when donor corneas from debilitated patients of ICU have been used for PKP. The reason claimed behind this is corneal storage mediums, especially Glutasol GS do not inhibit the growth of these organisms.

Recent studies have shown A. Baumannii drug resistance to ampicillin, chloramphenicol, tobramycin, amikacin and quinolones. Studies suggest that most effective preparation against A. Baumannii are meropenem,
imipenem and colistin. Colistin interacts with phospholipids and destroys cell wall. Topical and subconjunctival use of colistin has also been reported.

However, in our series we found maximal drug resistance to chloramphenicol (56.25%) followed by in decreasing order ceftazidime (43.75%), gentamicin (31.25%), amikacin (31.25%), Cefuroxime (18.75%), ofloxacin (18.75%), ciprofloxacin (6.25%), gatifloxacin (6.25%) and moxifloxacin (6.25%).

Case reports are available regarding A. Baumannii corneal infections to be presented with corneal perforations. In our series we found that 14.28% cases were presented with corneal perforations.

Secondary bacterial infections with Acinetobacter in a known case of herpes simplex viral keratitis has already been reported. In our case series, 12.50% patients were known cases of viral keratitis.

Cases of A. Baumanii keratitis have already been reported status post penetrating keratoplasty and steroids use. In our series, we found that 37.50% patients were status post PKP and using topical steroids.

Our case series is the largest case series of A. Baumannii keratitis which actually gives insight into different and unpredictable clinical picture produced by the same organism. Recognition of this organism as a pathogen, by other molecular diagnostic tools like PCR to detect the organism, may help in rapid diagnosis.

CONCLUSION

A. Baumannii produces multiple different types of clinical picture and high degree of clinical suspicion required in addition to excellent microbiology facility while dealing with routine common pathogens.

REFERENCES


Photodynamic Therapy (PDT) with Rosebengal Dye for Inhibition of Fungal Keratitis Isolates

Dr. Mukesh Taneja, Dr. Murthy Somasheila I, Prof. Jean-Marie Parel

Fungal keratitis (keratomycosis) is a potentially blinding disease affecting the cornea, which can cause severe visual loss if not treated at an early stage. Mycotic keratitis is more common in the tropical and subtropical locations than in the temperate regions. Its incidence has been reported to be between 1%-44%, of all microbial keratitis cases, depending on the geographic location. Incidence of fungal keratitis as a percentage of microbial keratitis, reported from tropical climates is 17% in Nepal, 36% in Bangladesh, 38% in Ghana and 35% in south Florida in USA. It constitutes about 44% of corneal ulcers in south India. In contrast, fungal keratitis generally accounts for only 1-5% of the keratitis in developed countries and temperate regions such as Britain, northern USA and Australia.

Fusarium species, followed by Aspergillus, are the most frequently isolated organisms from patients with fungal keratitis in tropical climates, whereas Fusarium and Candida are the common pathogens in more temperate areas.

Over the years, fungal keratitis has continued to be a cause of significant concern for the ophthalmologists. Despite all the advances made in the pharmaceutical research over the past few decades, we do not, still, have a very effective treatment options for ocular fungal infections. Treatment remains limited by scarcity of effective antifungals and poor ocular penetration of many existing medications. Resolution thus tends to be slow, and treatment duration can average 80 days or more and many a times patients have to undergo therapeutic penetrating ketatoplasty to save the eye.

Therefore, there is a definite need to develop new antifungal counter measures. One promising but underutilized, anti- fungal therapeutic modality, in this regard, is the light-based technology of photodynamic therapy (PDT).

Photodynamic therapy involves the use of a non-toxic light- sensitive dye called a photosensitizer (PS) combined with harmless visible light of the appropriate wave length to match the absorption spectrum of the PS. After photon absorption the PS reaches an excited state that can undergo reaction with ambient oxygen, resulting in the formation of reactive oxygen species (ROS) which then react with intracellular components and produce cell inactivation and death.

The little work that has been done regarding PDT for keratitis is limited, as of now, to the collagen cross linking (CXL) using Riboflavin and UV-A light,
that has been used in protocols, akin to the Dresden protocol for stabilizing keratectasia in patients having Keratoconus.

In vitro studies of CXL have found this treatment to be effective against certain common bacteria such as Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa but ineffective against Candida albicans, Fusarium and Acanthamoeba.

Limited number of clinical studies, done so far with Riboflavin and UV–A, have shown effectiveness for common bacteria but the results have been inconsistent for fungi and acanthamoeba. And that is the reason that this modality of PDT has not really been tried as a clinically effective tool for managing fungal keratitis or other microbial keratitis which are otherwise very difficult to treat with the currently available antimicrobial medications.

We decided to use Rose bengal dye for this purpose as this is routinely used in Ophthalmology clinics and is nontoxic to eye and some reports exist in literature regarding Rose bengal mediated photodynamic therapy for candida albicans in biofilms.

**MATERIALS AND METHODS**

Three fungi (Fusarium solani, Aspergillus fumigatus, Candida albicans) from patients with confirmed fungal keratitis were isolated and grown on Sabouraud-Dextrose agar plates. For the experiments, each isolate was suspended in sterile, deionized water and the concentration was adjusted with the photosensitizing agent or water depending on the experimental condition. The final concentrations for each organism were: F. solani - 5.5 x 10³/mL, A. fumigatus - 5.35 x 10³/mL, C. albicans - 3.8 x 10³/mL.

One milliliter of the isolate suspension was inoculated onto each plate for experimentation. Triplicate test plates were separated into 5 groups: Group 1 - no treatment, Group 2-0.1% RB alone, Group 3 – green light irradiation alone, Group 4 - Ribo PDT (Ribo+UVA), and Group 5 - RB PDT (RB+green). Irradiation was performed using a source housing a 518 nm green LED array and a 375 nm UV LED. A circular area was irradiated with a final energy density of 5.4 J/cm².

Plates were later placed in a 30º C non-CO² incubator and observed for growth. Images were taken daily to aid and document the fungi growth for analysis using a Labview program created in the laboratory.

**RESULTS**

RB PDT was the only successful growth inhibitor of all three fungal isolates in the irradiated area. Riboflavin PDT had no effect on the growth and the active and passive controls did not either. At day 3, the group 5 had
Figure 1: Growth of Candida Albicans for the different treatment groups at Day 3. Groups 1-4 grew with no bias and Group 5 has a clear inhibition zone in the irradiated area.

95.6% inhibition for C. albicans, 79.8% inhibition for A. fumigatus, and 78.2% inhibition for F. solani in the irradiated zones. All other groups grew without any bias (Figure 1).

DISCUSSION

The work outlined here was directed towards the development of Photodynamic therapy as a novel method for the treatment of Fungal Keratitis.

Antifungal PDT is an area of increasing interest, as research is now evolving to identify the photochemical and photophysical mechanisms involved in photoinactivation and to develop potent and clinically compatible photosensitizers and their delivery platforms.

There have been few reports on the use of PDT to kill yeasts and fungi in vitro. Effect of glutathione on rose bengal photosensitized Candida albicans was elucidated way back in 1993 by Lazarova et. al. In his paper in 1995, Paardekooper et. al. showed that photodynamic treatment of the yeast, Kluyveromyces marxianus with the sensitizer Toluidine blue leads to the loss of colony forming capability. Friedberg et. al. in 2001, demonstrated significant in vitro fungicidal activity against Aspergillus fumigatus of the photosensitizer Green 2W, activated with 630 nm light.

The little work that has been done regarding PDT for keratitis is limited, as of now, to the collagen cross linking (CXL) using Riboflavin and UV-A light, that has been used in protocols, akin to the Dresden protocol for stabilizing keratectasia in patients having Keratoconus.

In vitro studies of CXL have found this treatment to be effective against certain common bacteria such as Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa but ineffective against Candida albicans, Fusarium and Acanthamoeba.

Limited number of clinical studies, done so far with Riboflavin and UV-A, have shown effectiveness for common bacteria but the results have been inconsistent for fungi and acanthamoeba. And that is the reason that this modality of PDT has not really been tried as a clinically effective tool for
managing fungal keratitis or other microbial keratitis which are otherwise very difficult to treat with the currently available antimicrobial medications.

In our study we did not observe any growth inhibition with Riboflavin and UV irradiation for any of the three fungal isolates namely, Candida albicans, Fusarium solani and Aspergillus fumigatus. Martins et. al., also in 2008, in their paper reported that combined application of Riboflavin and UV-A light was ineffective in inhibiting the growth of Candida albicans. Kashiwabuchi et. al. also similarly reported that the impact of long-wave ultraviolet combined with riboflavin photosensitizer showed no antifungal effect on C. albicans and F. solani.

In contrast when Rosebengal dye was used as a photosensitizer and irradiated with green light in our study, we could see almost total zone of inhibition of growth for all the fungal isolates in all the Sab-Dex agar plates.

In comparison, no zone of inhibition of growth was seen in any of the agar plates in group 1 (no treatment), group 2 (application of RB only) and group 3 (irradiation with Green light only). That proves the fact that it is the photodynamic effect created by the photosensitization of Rosebengal dye following irradiation with green light that causes the growth inhibition of the fungal isolates and not any chemotherapeutic effect of the RB dye or phototoxicity of Green light.

We used this methodology of mixing the fungal isolates with Rosebengal dye and then plating that on Sab-Dex agar plates followed by irradiation with Green light as this comes close to the way that we envisage that photodynamic therapy might possibly be done in very near future for microbial keratitis in patients. This is somewhat akin to the way collagen crosslinking is done presently for patients with keratectasia using Riboflavin and UV-A light for photosensitization as per the Dresden protocol.

At the same time, the protocol that we have followed for this study, makes it very simple to perform these experiments repetitively with different microorganisms and at the same time, easy to interpret the results both qualitatively and quantitatively retaining complete objectivity at all times.

Rose Bengal dye was chosen for these experiments as it is one of the most commonly used dye in Ophthalmological clinics world over. Its safety for ocular use has been proven over the years and it’s use for photodynamic therapy for various microorganisms has been described in various studies.

Green light was used for irradiation as this provides the optimal spectrum of energy for photosensitization of Rose Bengal.

The model described presents an opportunity to study the effect of the UV-riboflavin combination in multiple micro-organisms, as well as determining the importance of riboflavin concentration for each tested strain.
CONCLUSION

The results of this study have very effectively demonstrated that in an in vitro environment, Photodynamic Therapy with Rose Bengal/ Green Light was effective against the clinical fungal isolates that we tested. In summary, of the experimental results, we can conclude the following:

1. Treatment with RB alone or Green Light was not successful in inhibiting the growth of fungal Isolates.
2. PDT with Rose Bengal and Green Light very effectively inhibited the growth of all the fungal isolates tested in this study, namely C. albicans, F. solani and A. flavus.
3. Riboflavin/UVA treatment did not show any effect against any of the fungal isolates tested.

This suggests that PDT with Rose Bengal and Green Light could be a way forward for treating refractive fungal corneal infections in patients. Since the results obtained in vitro do not always correlate with in vivo efficacy as there might be concerns regarding the absorption of RB and penetration of green light into the cornea for effective photoinactivation of microorganisms, therefore, further in-vivo and animal studies are under way to test the efficacy of this treatment for fungal keratitis, so as to evolve an optimal and safe protocol for PDT in patients.

REFERENCES


**Microsporidia Keratoconjunctivitis – Our Experience in A Tertiary Care Centre in North India**

Dr. Ravi Sharma, Dr. Uma Sridhar

Infective conjunctivitis due to viral or bacterial etiology is common the world over. Keratoconjunctivitis caused by the obligate intracellular pathogen such as microsporidia has been reported first in 1973 by Ashton et. al. Since then many publications have reported microsporidia keratoconjunctivitis from the world over in immunocompromised as well as in immunocompetent individuals. Most case series from the Indian subcontinent have been from South India. To the best of our knowledge, cases from the Northern part of India have been reported infrequently. Microsporidia are small (3.5-5.0μm in length by 2.0-3.0μm in width) oval obligate intracellular pathogens which can cause systemic involvement in human hosts in the form of sinus, gastrointestinal, pulmonary and renal
diseases. Microsporidia is usually opportunistic infectious pathogen in immuno compromised patients but can occur in immunocompetent patients as well. The route of transmission is unknown but thought to be either faeco-oral or by direct inoculation. Mainly two species of microsporidia i.e. Nosema and Encephalitozoon cause ocular infections. Earlier published reports of ocular microsporidiosis in the form of stromal keratitis in immunocompetent individuals commonly caused by Nosema and keratoconjunctivitis in those who were immunocompromised 2 commonly caused by Encephalitozoon is well known.

We report several cases of keratoconjunctivitis due to microsporidia from a tertiary care centre in North India.

MATERIALS AND METHODS

During a one year period between August 2012 to August 2013, 21 patients had been diagnosed clinically as having microsporidial keratoconjunctivitis. Most patients were diagnosed in the month of August which is the rainy season in North India. Three patients had been diagnosed in August 2012 and eighteen in August 2013. There were five female patients. Age ranges from seven years to eighty four years. Four patients had unilateral involvement. Of all cases suspected to be microsporidial keratoconjunctivitis, six patients underwent corneal scraping and Gram staining was done. All cases which were scraped showed the organism. There were seven cases in one family living together. Two members of this family were subjected to corneal scraping and had microsporidial spores in the smear.

Clinical diagnosis was based on history of getting wet in the rain, washing eyes with tap water, slit lamp showed conjunctival chemosis, coarse punctate lesions on the cornea and typical presence of “target” lesions on the cornea.

RESULTS

Out of 21 patients, 5 were female, with age ranging from 7 years to 84 years. Four had bilateral involvement, preauricular nodes enlarged in 16 cases, scraping done in 6 cases for Gram staining. Seven patients were from one household. All patients presented with coarse, multifocal, punctate epithelial keratitis with conjunctivitis. Three patients developed nummular opacities, keratic precipitates and anterior uveitis. All eyes were treated with topical fluoroquinolones and oral albendazole 400 mg once daily for one week and three patients with anterior uveitis received topical steroids. All cases resolved without visually significant sequelae.

DISCUSSION

Deep stromal keratitis and superficial punctate keratopathy are the main manifestations in the cornea. In immunocompetent patients it can
Cornea Free Papers

resemble herpes simplex viral keratitis. Conjunctival involvement may occur along with the keratitis. Keratoconjunctivitis mimicking adenoviral keratoconjunctivitis may occur especially in the rainy season. A mixed follicular and papillary reaction may occur in the conjunctiva. Sclera and uvea may also be involved.

In cases of keratoconjunctivitis, the presenting symptoms are photophobia, blurred vision and foreign body sensation. Conjunctiva usually is chemosed, with decreased luster. The corneal involvement is in the form of superficial punctate keratopathy with coarse fluorescein staining and non-staining epithelial opacities. The lesions are limited to the level of the epithelium and can be debrided.

Several case series describing keratoconjunctivitis in immunosuppressed and in later publications, immunocompetent patients have been published in the last two decades.\textsuperscript{3-6}

**Stromal Keratitis**

Stromal keratitis due to microsporidia is rarer than superficial keratoconjunctivitis. It occurs mainly in immunocompetent patients. The stromal infiltrates are usually middle to deep stromal. Stromal keratitis due to microsporidia can be mistaken for Herpes Simplex virus keratitis.

**CONCLUSION**

Microsporidial keratoconjunctivitis is usually unilateral, occurred mainly in males, presents as conjunctivitis and coarse, multifocal, punctate epithelial keratitis, and may cause anterior uveitis also. Management with topical fluoroquinolones with systemic albendazole is effective, with steroids for any associated anterior uveitis. Yee et. al.\textsuperscript{9} noted improvement with debulking and topical combined treatment with neomycin, bacitracin, and polymyxin B antibiotics, but complete resolution was achieved only after administration of systemic itraconazole signifying importance of systemic treatment also.

**REFERENCES**


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**Outcome of Medical Therapy for Microbial Keratitis**

Dr. Philip Aloysius Thomas, Dr. P Archana Teresa, Dr. Kaliamurthy, Dr. C A Nelson Jesudasen

Successful management of microbial keratitis requires prompt diagnosis and institution of appropriate antimicrobial therapy. Several classes of antibacterial agents (including aminoglycosides, cephalosporins and fluoroquinolones) are highly active against various bacterial pathogens. Fluoroquinolone drops are well-tolerated and can be administered using a convenient dosage schedule, when compared to fortified aminoglycoside and cephalosporin drops. Ciprofloxacin and ofloxacin are two commonly-used fluoroquinolones in the treatment of ophthalmic infections. Although ofloxacin is potent than ciprofloxacin against *Streptococcus pneumoniae* and some *Staphylococcus* species, ciprofloxacin is more effective against *Pseudomonas* species; moreover, ciprofloxacin is well-tolerated and effective in its commercially available strength. Natamycin (pimaricin), the first antifungal compound approved by the Food and Drug Administration of the United States for topical ophthalmic use, is the drug of choice for treatment of filamentous fungal keratitis in regions of the world where available.

In this paper, the efficacy of primary treatment with ciprofloxacin (0.3%) ophthalmic solution for proven or suspected bacterial keratitis, and ciprofloxacin (0.3%) solution and natamycin (5%) suspension for fungal keratitis was evaluated. Factors possibly influencing the outcome of medical therapy were identified.
MATERIALS AND METHODS

A prospective study of microbial keratitis (approved by the Institutional Ethics Committee) was conducted at Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamilnadu. Microbial keratitis was defined as loss of corneal epithelium with underlying stromal infiltrate and suppuration associated with signs of inflammation, with or without hypopyon. Ophthalmologists assessed patients using a standardized protocol and proforma. A detailed examination was performed on each patient at the slit-lamp; clinical features were documented, drawings were made in the patient’s record, and a photograph was taken of the affected eye. Microbiological specimens were collected as follows: scrapings from the ulcer were smeared onto two slides for microscopy (Gram stain and lactophenol cotton blue mount). Material was also inoculated directly onto various culture media. Bacteria and fungi were further identified using routine biochemical identification tests and selective media. Microbial cultures were considered to be significant by using standard criteria.5-7 If fungal hyphae were observed by microscopy, but failed to grow in culture, the causative organism was still reported as fungal.

In patients clinically diagnosed as bacterial keratitis topical ciprofloxacin (0.3%) was started; in patients clinically diagnosed as fungal keratitis, both topical ciprofloxacin and natamycin (5%) were started. The topical drops were instilled hourly during the day, and once every three hours at night.

Clinical outcomes were categorised as: (a) Treatment Success (complete healing of the ulcer within 21 days); (b) Slow Healing (progressive healing but incomplete ulcer epithelialisation at 21 days); (c) Indolent Ulceration (persistent epithelial defect unchanged in size after at least five days of primary treatment); d) Primary Treatment Failure (increase in ulcer size or infiltrate, necessitating other medical therapy but not surgery); or Penetrating keratoplasty (PKP) for rapidly increasing ulcer or infiltrate size. Analysis of putative factors influencing outcome of primary medical therapy were compared among the groups. Univariate and stratified analysis was performed to identify important risk factors. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 637 patients returned for one or more follow-up visit were recruited; their demographic and baseline clinical characteristics are presented (Table 1). None of the patients was debilitated or immuno compromised or had diabetes mellitus or human immunodeficiency virus. No patient had a history of contact lens use. Details of the aetiological agents are provided in Table 1 and the treatment regimens and the outcomes in Table 2.
Table 1: Demographic and clinical characteristics of all cases recruited into the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Followed-Up (N=637)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
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<tr>
<td>Sex (male)</td>
<td>336 (52.7%)</td>
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<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>0 – 20 years</td>
<td>73</td>
</tr>
<tr>
<td>21 – 40 years</td>
<td>237</td>
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<tr>
<td>41 – 60 years</td>
<td>259</td>
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<td>&gt;60 years</td>
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<tr>
<td>Microbial aetiology</td>
<td></td>
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<tr>
<td>Bacteria only</td>
<td>139</td>
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<tr>
<td>Fungi only</td>
<td>222</td>
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<tr>
<td>Mixed (bacteria and fungi)</td>
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<tr>
<td>Acanthamoeba</td>
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<tr>
<td>No growth</td>
<td>200</td>
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<tr>
<td>Clinical Features</td>
<td></td>
</tr>
<tr>
<td>Duration &gt;15 days</td>
<td>44</td>
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<tr>
<td>Ulcer &gt;6mm</td>
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<td>Ulcer depth &gt;50%</td>
<td>205</td>
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<tr>
<td>Hypopyon present</td>
<td>262</td>
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</table>

Table 2: Type of primary treatment as a possible determinant of outcome of microbial keratitis

<table>
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<tr>
<th>Outcome of microbial keratitis</th>
<th>Primary treatment regimen</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>Ciprofloxacin only</td>
<td>Ciprofloxacin and natamycin</td>
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<tr>
<td>Success</td>
<td>142</td>
<td>138</td>
</tr>
<tr>
<td>Healing</td>
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<td>60</td>
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<tr>
<td>Indolent ulceration</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Failure</td>
<td>57</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>304</td>
<td>266</td>
</tr>
</tbody>
</table>

Statistical analysis: $c^2 (d.f. = 6) = 19.26; \ p < 0.01$
Table 3: Significant Predictors for Indolent Ulceration in Patients with Microbial Keratitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients (%)</th>
<th>Risk Ratio</th>
<th>95% Confidence Intervals</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age &gt; 40 yrs. ≤ 40 yrs</td>
<td>36 / 327 (11.0)</td>
<td>1.63</td>
<td>0.9708 to 2.7207</td>
<td>χ² = 3.503 P = 0.0612</td>
</tr>
<tr>
<td>2. Ulcer largest diameter &gt; 6 mm</td>
<td>12 / 74 (16.2)</td>
<td>2.06</td>
<td>1.1435 to 3.725</td>
<td>χ² = 5.672 P = 0.0172</td>
</tr>
<tr>
<td>3. Ulcer smallest diameter &gt; 6 mm</td>
<td>8 / 37 (21.6)</td>
<td>2.69</td>
<td>1.375 to 5.2595</td>
<td>χ² = 7.981 P = 0.0047</td>
</tr>
<tr>
<td>4. Depth of ulceration &gt; 50% ≤ 50%</td>
<td>27 / 205 (13.2)</td>
<td>1.94</td>
<td>1.1825 to 3.1953</td>
<td>χ² = 7.029</td>
</tr>
<tr>
<td>5. Depth of infiltrate &gt; 60% ≤ 60%</td>
<td>37 / 300 (12.3)</td>
<td>2.06</td>
<td>1.2231 to 3.4684</td>
<td>χ² = 7.777 P = 0.0052</td>
</tr>
<tr>
<td>6. Hypopyon Present</td>
<td>42 / 262 (16.0)</td>
<td>4.43</td>
<td>2.4263 to 8.0771</td>
<td>χ² = 28.894 P = 0.0000</td>
</tr>
<tr>
<td>7. Severity Severe</td>
<td>32 / 251 (12.7)</td>
<td>2.07</td>
<td>1.2432 to 3.457</td>
<td>χ² = 8.15 P = 0.0043</td>
</tr>
</tbody>
</table>

The outcome of treatment by the type of primary treatment received is shown in Table 2. Factors that were significant predictors of indolent ulceration were a greatest or least diameter of corneal ulceration exceeding 6 mm, depth of ulceration exceeding 50%, depth of infiltration exceeding 60%, and the presence of a hypopyon. (Table 3).

Factors that were significant predictors of primary treatment failure were the above factors with, in addition, patient age exceeding 40 years, duration of symptoms exceeding 15 days and mixed bacterial-fungal growth, as opposed to bacterial growth alone (Table 4).

**DISCUSSION**

The previous studies have evaluated the clinical outcome of microbial keratitis. One such study dealt exclusively with bacterial keratitis, with the patients receiving ofloxacin therapy, while another such study dealt exclusively with fungal keratitis, with most of the patients receiving natamycin monotherapy. These two studies enrolled relatively smaller numbers of patients. The present study is unique in evaluating the efficacy
Table 4: Significant Predictors for Failure of Primary Medical Therapy (Primary Treatment Failure) in Patients with Microbial Keratitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients (%)</th>
<th>Risk Ratio</th>
<th>95 % Confidence Intervals</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age &gt; 40yrs.</td>
<td>52 / 327 (11.0)</td>
<td>1.90</td>
<td>1.2157 to 2.957</td>
<td>χ²=8.364, P=0.0038</td>
</tr>
<tr>
<td>2. Duration of symptoms &gt; 15 d</td>
<td>10 / 44 (22.7)</td>
<td>1.99</td>
<td>1.1042-3.5907</td>
<td>χ²=4.89, P=0.0270</td>
</tr>
<tr>
<td>3. Ulcer largest diameter &gt; 6 mm</td>
<td>23 / 74 (31.0)</td>
<td>3.16</td>
<td>2.0751 to 4.8262</td>
<td>χ²=27.381, P=0.0000</td>
</tr>
<tr>
<td>4. Ulcer smallest diameter &gt; 6 mm</td>
<td>15 / 37 (40.5)</td>
<td>3.84</td>
<td>2.4379 to 6.0538</td>
<td>χ²=29.039, P=0.0000</td>
</tr>
<tr>
<td>5. Depth of ulceration &gt; 50%</td>
<td>46 / 205 (22.4)</td>
<td>3.0</td>
<td>1.9732 to 4.5647</td>
<td>χ²=28.722, P=0.0000</td>
</tr>
<tr>
<td>6. Depth of infiltrate &gt; 60%</td>
<td>53 / 300 (17.7)</td>
<td>2.36</td>
<td>1.5061 to 3.6989</td>
<td>χ²=15.185, P=0.0000</td>
</tr>
<tr>
<td>7. Hypopyon Present</td>
<td>44 / 262 (16.8)</td>
<td>2.08</td>
<td>1.3377 to 3.231</td>
<td>χ²=11.092, P=0.0008</td>
</tr>
<tr>
<td>8. Aetiology Mixed Bacteria only</td>
<td>7 / 34 (20.6)</td>
<td>2.6</td>
<td>1.0896 to 6.2116</td>
<td>χ²=4.708, P=0.0300</td>
</tr>
<tr>
<td>9. Severity Severe</td>
<td>45 / 251 (17.9)</td>
<td>2.03</td>
<td>1.3353 to 3.0917</td>
<td>χ²=11.399, P=0.0007</td>
</tr>
</tbody>
</table>

Of a commonly used primary therapeutic regimen for microbial keratitis, namely topical ciprofloxacin and topical natamycin for fungal keratitis, and topical ciprofloxacin monotherapy for bacterial keratitis and for suppurative keratitis of unknown aetiology, in a large number (> 600) of patients.

Of the 637 patients, 570 patients received topical ciprofloxacin, alone or in combination with topical natamycin, as primary therapy for suppurative (suspected non-viral microbial keratitis) while 67 patients received other forms of primary therapy. In the 570 patients, 73% exhibited a favourable response to therapy (complete resolution or marked improvement of keratitis), and 27% did not (keratitis remained unchanged or progressed).

The influence of gender on outcome of keratitis has not been reported. In the present study, there was a favourable response to therapy in 242 (72%) of
336 male patients, which was not significantly different from the 217 (72%) of 301 female patients who exhibited a favourable response to therapy.

Age has been reported to be an important determinant of the outcome of microbial keratitis. The results of the present study support this concept: favourable responses were observed in 86% of patients aged 1 to 20 years, 73% in those aged 21 to 60 years and 49% in those who were older than 60 years (p < 0.001). The percentage of patients exhibiting complete resolution of keratitis decreased from 63% to 31% with increasing age while the percentage of those with progression of keratitis or penetrating keratoplasty done increased from 10% to 31% with increasing age.

The duration of symptoms before presentation for treatment seems to be an obvious variable that would influence the ultimate outcome of keratitis. In the present study, a favourable response to therapy was observed in 434 (74%) of 587 patients who had symptoms for 1 to 15 days, in 17 of (53%) of 32 patients who had symptoms for 16 to 30 days and in 4 (33%) of 12 patients who had symptoms for more than 30 days differences which were statistically significant (p<0.001).

REFERENCES
Omega 3 Fatty Acids in Dry Eye Syndrome. are they Really Effective?

Dr. Rahul Bhargava, Sidharth Kaushal, Prachi Kumar, Sidharth Kaushal

Dry eye is increasingly being recognized as a growing public health problem. There have been significant advances in our understanding of its pathogenesis and ocular surface inflammation is now considered an integral part of dry eye disease.¹

Ocular surface health may be influenced by hormones, contact lens use, refractive surgeries, humidity, medications, smoking and usage of computers.²,³,⁴,⁵

Role of VDTs in every aspect of life (school, office and home) has increased exponentially. Almost everyone including children, college students, software professionals and even the elderly are hooked on to computers every day, ranging from 2 to 12 hours. Usages of mobile phones further add to the overall burden. Prolonged visual display terminal tasks reduce blink rate, blink amplitude and blink quality leading to tear film instability.⁶,⁷ People experience one or more symptoms referred to as computer vision syndrome; these include eye strain, tired eyes, headache, burning of eyes, redness, foreign body sensation, blurring of vision, sometimes accompanied by backache and neck pain; visual symptoms predominate in 64-90% patients.⁸,⁹,¹⁰

Artificial tear supplementation, the most commonly used therapy for dry eye however provides incomplete and asymptomatic relief and do not reverse metaplastic changes.¹¹,¹²,¹³

Treatment of dry eye has undergone a paradigm shift in strategy which also includes behavioural and environmental modifications like learning to take breaks while reading, changing angulation of computer monitors to decrease size of lid apertures, and changing ambient humidity conditions in work environment.¹⁴

O3FAs are anti-inflammatory and have been proven to be effective in conditions like rheumatoid arthritis and coronary artery disease.¹⁵ However, safety and efficacy of O3FAs for dry eye in VDT users has not been documented. An extensive review of literature (Medline search) revealed that no randomized trial has been done to determine this.

In the present study, we hypothesize that oral O3FA supplementation will improve ocular symptoms, limbal cytology and morphology (as seen on conjunctival impression cytology) as well as clinical markers (Schirmer-1 test values and fluorescein tear break-up time (TBUT) in VDT users with dry eye symptoms when compared to administration of placebo.
MATERIALS AND METHODS

A prospective, multi-centric, randomized, double blind interventional study was done at three referral eye centers. The trial was approved by the institutional review boards and the local ethics committee. A written informed consent was obtained from all patients willing to participate in the study based on Helsinki protocol.

Inclusion criteria

Symptomatic patients with VDT use of more than 6 hours a day for minimum one year participated in the study. The patients were enrolled on the basis of a questionnaire of dry eye related symptoms (Table 1) (Dry Eye Scoring System, DESS ©).  

Exclusion criteria

Patients with current ocular infection, patients on tetracycline, corticosteroids or anti-glaucoma medications; past history of laser in situ keratomileusis (LASIK), herpetic eye disease, diabetes, liver diseases were excluded. Other exclusion criteria included pregnancy or lactating mothers, HIV and Hepatitis B and C. Patients with inability to swallow soft gel capsules, on aspirin or anti-coagulant therapy, and those allergic to fluorescein were also excluded. All topical medications and contact lens usage was discontinued 3 weeks prior to intervention.

Randomization, masking and sample size calculation.

To calculate sample size to compare the mean difference in symptom scores between the two groups, a pilot study was first done on 25 subjects. The mean decrease in symptoms score in test group was 0.79 and in placebo group 0.67, respectively. The common standard deviation was 0.45. Assuming 1:1 randomization, alpha was set at 0.05 and power 90%. The estimated sample size in each group was 237. Figure 1 shows the patients flow chart, randomization schedule and follow up protocol.

Patients were randomly allocated to one of the two groups by parallel assignment. The allocation codes were generated by a DOS based software in the department of Community Ophthalmology. The codes were sealed in blue colored envelopes and were opened by health care personnel not involved in patient care. Group 1 received two 300 mg capsules containing each containing 180 mg Eicosapentaenoic acid (EPA) and 120 mg Docosahexaenoic acid (DHA), twice daily for 3 months. Group 2 received two capsules containing olive oil, twice daily for 3 months. The subjects were masked to the contents. The two types of capsules and packs were similar to each other. The subjects were instructed to return the bottles at month visit, when another pack with 120 capsules were provided to them.
Outcome Measures

Patients were seen at baseline, 1 month, 2 months and 3 months after dietary supplementation. The primary outcome measure were decrease from baseline in subjective dry eye related symptoms at 3 months post-intervention. A score of 0-3 was assigned to dry eye related symptoms like ocular fatigue, blurring of vision, itching or burning, sandy or gritty sensation and redness respectively (DESS ©). When absent (0), sometimes present (1), frequently present (2), and always present (3). A score of 0-6 was mild, 6.1-12 moderate and 12.1-18, severely symptomatic dry eye (Table 1).

The secondary outcome measures after 3 months of intervention were change in Schirmer's 1 test for tear production, TBUT as a measure of tear film stability and conjunctival impression cytology (CIC) scores for cellular morphology and goblet cell density.

Ocular Examination and Tear Function Tests

At each follow up visit, the subject underwent a detailed ocular examination by an independent investigator (not a study surgeon, SK). This included recording of corrected distance visual acuity (CDVA), and slit lamp examination; this included assessment of lid margins, eye lashes, and Meibomian gland orifice for any blockage or occlusion.

At each examination, subjects underwent tests of tear film characteristics as Schirmer’s, tear break-up time (TBUT), and ocular surface parameters (conjunctival impression cytology (CIC)). One eye from each patient was selected at random for evaluation. Furthermore, the subjects were given dry eye questionnaire at each visit. The independent investigator (SR) was masked to the information obtained from the questionnaire.

TBUT was first performed as eyelid manipulation may adversely influence the results. Three readings were taken in succession and averaged. The subject then waited for 30 minutes and Schirmer’s test with anaesthesia was done with eyes closed.

The eye was anaesthetized with one drop of 4% Xylocaine. The lacrimal lake at inner canthus was dried with a cotton tip applicator. A circular 0.22 micron filter paper measuring 13 mm in diameter (Sartorius, Germany) was grasped with a blunt tipped forceps and applied over the inferior bulbar conjunctiva. The paper strip was gently pressed with a glass rod held in the other hand. The filter paper was removed in a peeling fashion after 4-10 seconds and specimen transferred to the lab for staining and fixation. The filter paper was placed on a glass slide with albumin paste for specimen transfer. The slide was labelled and numbered, and then it was stained with periodic acid-Schiff and counterstained with haematoxylin and eosin. The mounted slide was first examined under the microscope with 100X low
power field (LPF). After localization, cells were then analyzed with 400X HPF magnification. At least 10 HPF were examined for goblet cells and epithelial cells as there is variability in GCD estimates across CIC samples. Grading and scoring was carried out by criteria suggested by Nelson. Nelson Grades 0 and 1 were regarded as normal, whereas grades 2 and 3 were considered to represent abnormal cytology.

**Statistics**

Statistical analysis was performed on an intent to treat basis using SPSS software for windows (version 18, SPSS Inc.). Means of groups were compared using t-tests. Chi-square tests were used for proportions. A p-value <0.05 was considered statistically significant.

**RESULTS**

A total of 478 eyes of patients were enrolled in the study. Out of these 220 patients were randomized to O3FA group and 236 eyes to the placebo group. Four hundred and fifty six patients completed the 3 months follow up. Ten patients were lost in follow up and another six excluded due to faulty impression cytology slides. Six patients were excluded due to adverse effect of gastric intolerance to omega 3 fatty acids.

The mean age was 22.80±5.48 years in the O3FA group and 23.67±6.85 years in the placebo group, respectively (P=0.134). Overall, 219 males and 237 females participated in the study.

Table 2 shows that baseline test values were comparable in O3FA and placebo groups. In O3FA group, 74 (32.4%) were mild, 142 (62.3%) moderate and 12(5.3%) severely symptomatic at baseline. Likewise, in the placebo group, 85(37.3) were mild, 138(60.5%) moderate and 5(2.2%)0 severely symptomatic, respectively. The mean symptom score between both groups was comparable (P=0.028).

In the O3FA group, 32.9% patients had abnormal Schirmer’s in comparison to 30.7 % patients in the placebo group at baseline. The mean Schirmer’s test values between the groups was comparable (P=0.632).

In O3FA group, 54.8% patients had abnormal TBUT as compared to 56.3% patients in the placebo group at baseline. The mean TBUT scored between the groups were comparable (P=0.023). Similarly, 35.5% patients in O3FA group had abnormal cytology (Nelson grades 2 and 3) as compared to 33.8% patients in the placebo group. The mean CIC values were comparable in both the groups at baseline (P=0.013). Table 3 shows grade wise (Nelson) CIC scores in both the groups.

Table 4 shows mean test values at 3 months following dietary intervention. The change in subjective symptom score was compared in the O3FA group
Table 1: Dry eye questionnaire and scoring system (DESS©)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Absent (0)</th>
<th>Sometimes (1)</th>
<th>Frequent (2)</th>
<th>Always present (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itching or burning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandy or gritty sensation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blurring of vision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular fatigue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excessive blinking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scores of 0 to 6 were mild, 6.1 to 12 were moderate, and 12.1 to 18 indicated severely symptomatic dry eye [11]. © Bhargava R. Laser Eye Clinic, Noida, India.

Table 2: Mean baseline characteristics of Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Omega 3 group</th>
<th>Placebo group</th>
<th>P-value (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom score</td>
<td>8.00±2.59</td>
<td>7.52±2.05</td>
<td>0.028</td>
</tr>
<tr>
<td>Schirmer's score (mm)</td>
<td>20.13±4.20</td>
<td>19.93±4.72</td>
<td>0.632</td>
</tr>
<tr>
<td>TBUT (seconds)</td>
<td>11.74±1.65</td>
<td>11.54±2.03</td>
<td>0.238</td>
</tr>
<tr>
<td>CIC (BL)</td>
<td>1.22±0.81</td>
<td>1.02±0.91</td>
<td>0.013</td>
</tr>
<tr>
<td>GCD (cells/mm²)</td>
<td>890±336</td>
<td>875±383</td>
<td>0.652</td>
</tr>
</tbody>
</table>

Table 3: Grade wise Conjunctival Impression cytology scores

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Baseline Conjunctival Impression Cytology (CIC) scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Grade 1</td>
</tr>
<tr>
<td>Omega 3 group (N, %)</td>
<td>42(18.4)</td>
</tr>
<tr>
<td>Placebo group (N, %)</td>
<td>82(36)</td>
</tr>
</tbody>
</table>

Table 4: Mean test values at 3 months post intervention

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Omega 3 group</th>
<th>Placebo group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom score</td>
<td>3.91±2.17</td>
<td>6.81±2.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Schirmer's score (mm)</td>
<td>21.36±4.00</td>
<td>20.53±4.67</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>TBUT (seconds)</td>
<td>14.98±1.76</td>
<td>11.98±2.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIC (BL)</td>
<td>0.54±0.65</td>
<td>0.88±0.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCD (cells/mm²)</td>
<td>1018±281</td>
<td>899±375</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 5: Mean change in test values post intervention

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Omega 3 group</th>
<th>Placebo group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom score</td>
<td>4.09±2.85</td>
<td>0.71±1.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Schirmer's score (mm)</td>
<td>1.23±1.17</td>
<td>0.65±0.81</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>TBUT (seconds)</td>
<td>3.24±1.76</td>
<td>0.44±0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIC (BL)</td>
<td>0.44±0.62</td>
<td>0.14±0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCD (cells/mm²)</td>
<td>127±117</td>
<td>24±70</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
and placebo groups. In the O3FA group, at 3 months, 159 (70%) patients were symptoms free, 54 (23.7%) continue to experience mild symptoms and 15(6.58%) patients were moderately symptomatic. In the placebo group, 34(14.9%) were symptom free, 78(34.2%) continue to experience mild symptoms. However, 113(49.56%) patients were moderately symptomatic and 3(1.32%) severely symptomatic after intervention. The mean decrease in symptom score in O3FA group was 4.09±2.85 as compared to 0.71±1.37 in the placebo group (t test, P<0.001).

The mean increase in Schirmer’s score was 1.33±1.17 mm as compared to 0.65±0.81 mm in the placebo group (t test, P=0.008).

The mean increase in TBUT at 3 months was 3.24±1.76 seconds as compared to 0.44±0.62 seconds in the placebo group (Table 5).

There was a significant improvement in CIC scores in O3FA group as compared to the placebo group. After 3 months of treatment with O3FAs, the mean improvement in CIC scores was 0.44±0.62 as compared to 0.14±0.42 in the placebo group (P<0.001); the main cytological feature being presence of smaller cells with both polygonal and round shape and increase in goblet cell counts; the mean increase in goblet cell density in O3FA group was 127±117 cells per mm² as compared to 24±70 cells per mm² in placebo group (P<0.001).

**DISCUSSION**

The role of visual display terminals (VDTs) and internet has increased tremendously in our day to day life. Most jobs are now computer dependent and people have begun to spend more time in front of the computers at work and at home. This spurt has led to an increase in dry eye related symptoms in young and middle aged individuals and office going population.  

The clinical diagnosis of dry eye has been a challenging task for ophthalmologists due to lack of a universally accepted gold standard test. Therefore, there has been a shift towards symptom based assessment as a key component of clinical diagnosis. Moreover, there is lack of correlation between ocular symptoms and signs observed; patient may not be symptomatic despite abnormal tear function tests and not all symptomatic patients have abnormal tear function tests. This was reinforced by observation in present study that amongst symptomatic patients with dry eyes, 33% had abnormal Schirmers, 55% had abnormal TBUT and 35 % abnormal cytology at baseline in both groups combined.

The present study assessed whether dietary supplementation of O3FAs cause improvements in symptoms, clinical parameters, epithelial morphology and goblet cell density in VDT users as compared to administration of a placebo.
One of the most important features of dry eye syndrome is alteration of conjunctival and corneal epithelium as seen on vital staining. However, these methods do not indicate degree of squamous metaplasia or changes in goblet cell density. Therefore, in the present study, we used impression cytology scores as a direct indicator of ocular epithelial damage and subsequent improvement following dietary intervention.

Although dryness of eye has been more prevalent elderly women, preponderance of dry eye in young females in present study highlight the increasing impact of VDT on dry eye related symptoms. O3FA dietary supplementation significantly reduced symptoms in the test group (4.09±2.85) as compared to placebo group (0.71±1.37); seventy percent patients were totally symptom free in contrast to 14.9 % patients in the placebo group at conclusion. In a double blind clinical trial, Kangari et. al. found that even short term (n=30 days) consumption of O3FAs was associated with improvement in dry eye associated symptoms.

At three months, mean Schirmer’s score improved from baseline in both the groups (1.23±1.17 versus 0.65±0.81 mm); however, the magnitude of improvement was relatively small (P=0.008). This finding was similar to a multi-centric, randomized controlled double blind trial by Bhargava et. al. in which the authors found a small drift in Schirmer’s scores and a slight increase in overall average at 3 months post intervention. However, another randomized controlled trial by Wojtowicz et. al. found increased tear production and tear volume following dietary intervention with O3FAs for three months. Small sample size of patients in this study (n=36) could explain difference in observation.

There was a significant improvement in TBUT in O3FA at three months. A mean increase in TBUT of 3.24±1.76 seconds was seen in O3FA group in contrast to 0.44±0.62 seconds in placebo group (P<0.001). Impression cytology scores improved significantly from baseline in the omega 3 group as evidenced by increased goblet cell counts and smaller polygonal epithelial cells. In a multi centre, double-masked, randomized, controlled trial assessing the effect of oral supplementation of O3FAs and O6FAs on a conjunctival inflammatory marker in dry eye patients, Brignole-Baudouin et. al. found that supplementation with omega-3 and omega-6 fatty acids can reduce expression of HLA-DR conjunctival inflammatory marker on CIC and may help improve DES symptoms.

The significant improvement in dry eye related symptoms and TBUT scores in O3FA group suggests that O3FA dietary supplementation improves inherent tear film stability rather than increasing tear volume and production. This observation was further reinforced by decreased tear evaporation rates improved CIC scores in O3FA group.
Dietary consumption of O3FAs causes significant improvement in dry eye related symptoms, decreased tear evaporation rates and better impression cytology scores as evidenced by increased goblet cell density and improved cellular morphology in VDT users. In conclusion, O3FAs are really effective.

**REFERENCES**


**A Noval Approach to Test Tear Film Stability**

**Dr. Vipul Bhandari**, Dr. Jagadeesh Kumar Reddy K, Dr. Piyush Gupta, Dr. Siddharthan

Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tears film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Dry eye evaluation includes tests for assessment of tear production, tear film stability, ocular surface disorder, and various laboratory and
histological tests. A study by Serin D et. al. ranked tear break up time (93%), corneal staining (85%), tear film assessment (76%), conjunctival staining (74%), and the Schirmer test (54%) as the most commonly used diagnostic tests for initial assessment of dry eye. The various non-invasive methods that have been developed to assess tear film stability are by observing standard keratometer mires or a grid pattern projected onto 70-80% of the corneal surface by using a xeroscope (modified bowl perimeter), modified keratometer mires with a HIR-CAL grid, a hand-held keratoscope with a Loveridge grid. These instruments all project a white grid on a black background. The Keeler Tearscope and Keeler Tearscope Plus (Keeler Ophthalmic Instruments) with a corneal topography grid attachment project a black grid on a white background. The Keeler Tearscope and Keeler Tearscope Plus project a black grid on a white background. The Keeler Tearscope and Keeler Tearscope Plus project a black grid on a white background. Tear film lipid layer interferometry is another method that has been described to measure the superficial lipid layer of the tear film.

The majority of established tear film examination methods to date are based on observation of induced tear film reflections. We selected Optikon Keratron Scout as an aid in diagnosis of dry eye.

**MATERIAL AND METHODS**

This was a prospective comparative study of 25 subjects (50 eyes) with dry eye attending a tertiary eye care clinic in South India and 25 normal control subjects (50 eyes) recruited from a population based study. Written informed consent was obtained from all the subjects after the approval of the Scientific and the Ethical committee. All subjects were interviewed regarding their medical history. The diagnosis of dry eye was made based on Ocular Surface Disease Index (OSDI) questionnaire. It has 12 items; 6 for visual function, 3 for ocular symptoms and 3 for environmental triggers. It gives a score from 0 to 100; 100=complete disability; 0=no disability. In this study the Optikon Keratron Scout; (Optikon, Italy), a topographer, was used in the evaluation of dry eyes by studying the irregularities in the mires of the Placido’s disc that is reflected onto the cornea. All patients diagnosed with dry eyes were subjected to non-invasive tear break up time (NI-TBUT) using the Optikon Keratron Scout topographer (Optikon, Italy). The instrument shoots 15 high speed (one picture per second) photographs of the cornea with a projection of placido’s disc on the corneal surface (Figure 1,2,3,4,5,6,7). These pictures are then analysed for irregularities in the mires. The method adopted to identify the irregularity in the mires was the appearance of discontinuity in the mires in central 6 mm mire. It is different from distortion of the image. Presuming that the tear film break up time is 10 seconds, any patient showing irregularities in the mires before the tenth picture were
considered as dry eyes. Slit-lamp examination, TBUT measurement (2 mL of a preservative-free combination of 1% fluorescein dye), and the Schirmer I test was performed. The gap between the tests was more than 15 minutes. In TBUT we used a slit lamp with a cobalt blue light source to view the tear film after the instillation of fluorescein. The first appearance of a dark spot marks the tear break-up time and this time was recorded. A tear film break-up time of less than 10 seconds indicates instability. Schirmer’s I test was done using Whatman filter paper with a scale 0-35 mm. Patients were asked to sit in a room devoid of any air conditioning or fan and were asked to blink normally. A cut off of 5.5 is considered significant.

Statistics

Pearson correlation test and paired t test were carried out for doing the statistics.

RESULTS

A total of 50 eyes of 25 dry eye patients (15 females and 10 males) and 50 eyes of 25 normal control subjects (15 females and 10 males) were recruited for the study. All subjects were of Indian origin (Table 1). Mean TBUT was 7.56 s in the study group and 12.92 s in the control group mean NIBUT was 5.78 s in the study group and it was 11.66 s in the control group. Mean Schirmer-I was 37.44 in study group and 29.26 in control group. Mean OSDI score was 37.54 in the study group and 5.497 in control group. There was a significant difference (p<0.02) between the TBUT and NI-BUT values in the case group as well as in the control group (p<0.01). The values of NI-BUT were significantly lower than the values of TBUT in both the groups. There was a significant difference in the NI-BUT values for cases and controls (Table 2). Spearman correlation analysis showed that the values of NI-BUT in the study group correlated well with OSDI score (ρ=-0.85, P<0.001), Schirmer

### Table 1: Showing age between Case and Control

<table>
<thead>
<tr>
<th></th>
<th>Case-Age</th>
<th>Control-Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>42.64</td>
<td>42.24</td>
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<tr>
<td>Known Variance</td>
<td>172.64</td>
<td>182.8</td>
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<tr>
<td>Observations</td>
<td>50</td>
<td>50</td>
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<td>Hypothesized Mean Difference</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>P(Z&lt;=z) one-tail</td>
<td>0.44</td>
<td></td>
</tr>
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<td>z Critical one-tail</td>
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<td></td>
</tr>
<tr>
<td>P(Z&lt;=z) two-tail</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>z Critical two-tail</td>
<td>1.96</td>
<td></td>
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### Table 2: Relation between TBUT and NI-BUT

<table>
<thead>
<tr>
<th>Cases</th>
<th>TBUT</th>
<th>NI-TBUT</th>
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<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>7.56</td>
<td>5.78</td>
</tr>
<tr>
<td><strong>Known Variance</strong></td>
<td>16.25</td>
<td>14.13</td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>Hypothesized Mean Difference</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>2.28</td>
<td></td>
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<tr>
<td><strong>P(Z&lt;=z) one-tail</strong></td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><strong>z Critical one-tail</strong></td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td><strong>P(Z&lt;=z) two-tail</strong></td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><strong>z Critical two-tail</strong></td>
<td>1.96</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Controls</th>
<th>TBUT</th>
<th>NI-TBUT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>12.92</td>
<td>11.66</td>
</tr>
<tr>
<td><strong>Known Variance</strong></td>
<td>2.48</td>
<td>2.15</td>
</tr>
<tr>
<td><strong>Observations</strong></td>
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<td>50</td>
</tr>
<tr>
<td><strong>Hypothesized Mean Difference</strong></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>4.14</td>
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<tr>
<td><strong>z Critical one-tail</strong></td>
<td>1.64</td>
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<tr>
<td><strong>P(Z&lt;=z) two-tail</strong></td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td><strong>z Critical two-tail</strong></td>
<td>1.96</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Case-NI-TBUT</th>
<th>Control-NI-TBUT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>5.78</td>
</tr>
<tr>
<td><strong>Known Variance</strong></td>
<td>14.13</td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td>50</td>
</tr>
<tr>
<td><strong>Hypothesized Mean Difference</strong></td>
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<tr>
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<td>1.64</td>
</tr>
<tr>
<td><strong>P(Z&lt;=z) two-tail</strong></td>
<td>0.00</td>
</tr>
<tr>
<td><strong>z Critical two-tail</strong></td>
<td>1.96</td>
</tr>
</tbody>
</table>

### Table 3: Paired Samples Test

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Ig. (2-tailer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Pair: RE_TBUT-RE_N</td>
<td>1.640</td>
<td>1.497</td>
<td>.299</td>
<td>1.022</td>
</tr>
<tr>
<td>Pair: LE_TRUT-LR_N</td>
<td>1.920</td>
<td>1.706</td>
<td>.341</td>
<td>1.216</td>
</tr>
</tbody>
</table>
values ($\rho = 0.834, P<0.001$), TBUT values ($\rho = 0.919, P<0.001$). Similarly, the NI-BUT values in the control group showed correlation with TBUT values ($\rho = 0.783, p<0.001$). But there was no significant correlation between NI-BUT values and ODSI score in controls. NI-BUT was found to have a sensitivity of 96% and specificity of 80% when the cut off was kept at 10 seconds. The positive predictive value was found to be 82.76%, and negative predictive value was found to be 95.24%. In comparison, TBUT was found to have a sensitivity as well as specificity of 92% when the cut off was kept at 10 seconds. The positive predictive value was 92% and negative predictive value was 92%.

**DISCUSSION**

The diagnosis and management of Dry Eye Disease is a challenge in the modern ophthalmologic clinical practice. A large number of patients present with dry eye symptoms, and require a high standard of care. It is not always easy to objectively quantify dry eye disease. The ophthalmologist is equipped with an arsenal of tests and questionnaires that are substantially subjective and aren’t necessarily reproducible nor definitive. The NI TBUT quantifies both anatomical and physiological function as the deterioration of the mires quality directly affects the visual function by reduction in the quality of image formed at macula. The TBUT values are found to be comparable to the NIBUT values and as such either of the two techniques can assuredly be used to assess the tear film stability.\(^\text{15}\) We recommend using non invasive technique of measuring tear film break up time with Optikon keratron scout topographer as a reliable objective tool to identify dry eye disease. Using this system we found that the values of NI-BUT were significantly lower than those of FBUT ($p<0.001$) in all the subjects tested. This finding is similar to Gumus \textit{et. al.}\(^\text{16}\) and Hong \textit{et. al.}\(^\text{17}\) but different from Mengher \textit{et. al.}\(^\text{18}\). Notably, our study found that the NI-BUT measured by this newly developed technique showed good correlation with the FBUT. Cho and Douthwaite did not find good agreement between FBUT and NI-BUT\(^\text{19}\). In our study, the NI-BUT was significantly decreased in dry eye patients compared with normal subjects ($p<0.001$). This study had the advantage that it also considered clinical dry eye grading as compared to previous studies. Moreover, this study recruited patients from the entire spectrum of dry eye disease, from mild to severe dry eye. The study shows significant correlation between the clinically obtained subjective ODSI questionnaire score and objective testing by NI-TBUT in cases of dry eye. Thus, NI-BUT can be a valuable tool in diagnosis of dry eye and its severity as a quick, non invasive and inexpensive test. The sample size was relatively small and Reflex tearing during delayed blinking may have an effect on tear break up times. We investigated the performance of a non invasive technique for
measuring tear film stability to aid in the diagnosis of dry eye disease. It is a useful non invasive objective method for the detection of dry eye and may be useful in monitoring the efficacy of therapies for dry eye. More studies are required to validate our findings.

REFERENCES

Combined Insitu Conjunctivoplasty and Conjunctival Autograft–Future in Double Head Pterygium Surgery

Dr. Ashok Kumar Meena

Ptterygium is a common ocular surface disorder characterized by a wing-shaped over growth of the bulbar conjunctiva over the limbus, often nasally. The presence of both nasal and temporal pterygia in the same eye, termed double-headed pterygia, is rare with reported incidence rate of less than 2.5%.\textsuperscript{1} Pterygium excision with conjunctival autograft (PECA) is a widely performed procedure for single-headed pterygia, with recurrence rates varying from 2% to 39%.\textsuperscript{2,3} The management of double-headed pterygia poses a significant challenge because a large amount of conjunctival donor tissue is required to cover both bare sclera defects. Various surgical techniques have been described to address the paucity of conjunctival donor tissue in cases of double-headed pterygia, where as also reducing the risk for recurrence. Some of these techniques include the following in combination with conjunctival autograft: amniotic membrane transplantation,\textsuperscript{4} splitgraft,\textsuperscript{5} bare sclera,\textsuperscript{6} conjunctival rotational autograft combined,\textsuperscript{7} and Sequential pterygium excision with conjunctival autograft.\textsuperscript{8} The purpose of this study is to evaluate the efficacy and safety of in situ-conjunctivoplasty combined with conjunctival autograft.

MATERIALS AND METHODS

A prospective, non comparative, interventional case series approved by institutional research ethics board and informed consent was obtained from all patients.

Patient with double head pterygium underwent pterygium excision, followed by insitu conjunctivoplasty combined with conjunctival autograft

between September 2013 to November 2013 at a tertiary eye centre in central India.

Primary and secondary outcome noted were recurrence and complication respectively.

40 eye of 40 consecutive patients with primary double pterygium were recruited.

Inclusion criteria included patients with primary double-head pterygium, ≥18 years of age, and able to cooperate for surgery under peribulbar anesthesia.

Patients with recurrent pterygium, scarred superior conjunctiva, history of glaucoma, and cicatricial ocular surface disease were excluded.

Recurrence rate was defined as any fibrovascular growth measuring ≥1 mm across the limbus on to the cornea. Patients were followed at 1 month and 6 month.

Preoperatively, uncorrected visual acuity (UCVA) and best-corrected visual acuity (BCVA), manifest refraction, and intraocular pressure (IOP; by Goldmann appplanation tonometry) were recorded. Slit-lamp photography was taken for documentation. All operations were performed by 1 surgeon under peribulbar anesthesia by using lignocaine hydrochloride 2% with hyaluronidase.

**Surgical procedure**

Under peribulbar anaesthesia, after painting and draping, a wire speculam was used to separate the lids. The head of the smaller pterygium was grasped with toothed forceps, lifted off the corneal surface and excised. The subconjunctival fibrovascular tissue was dissected with the spring scissors, followed by in situ conjunctivoplasty done by suturing the conjunctiva with 8-0 vicryl. The fibrovascular tissue of the larger pterygium was dissected of the cornea and sclera. The bare sclera defect was covered with conjunctival autograft harvested from the superior bulbar conjunctiva with the limbal side of the graft apposed to the limbus of the recipient site and anchored in place with single 8-0 vicryl suture. Postoperatively patient received a tapering dose of antibiotic steroid eye drop, lubricant eye drops.
RESULTS

40 eye of 40 consecutive patients underwent combined insitu conjunctivoplasty and conjunctival autograft in primary double head pterygium patients. 34 out of 40 patients came to follow-up for 6 month.

<table>
<thead>
<tr>
<th>Demographic data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>28</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
</tr>
<tr>
<td>Mean age</td>
<td>47.36±13.84 year</td>
</tr>
<tr>
<td>Mean follow-up</td>
<td>4.67±1.37 month</td>
</tr>
</tbody>
</table>

Recurrence was seen in two eyes, no intraoperative and postoperative complication was seen. mean BCVA, which does not change after surgery.

DISCUSSION

PECA is considered to be the gold standard in the management of primary pterygia and is associated with the lowest recurrence rates. 10 This in combination with the use of fibrin tissue glue in stead of sutures has provided a further reduction in recurrence rates for primary pterygia.9,11,12 Because of the lack of available donor conjunctival tissue, a single procedure involving the excision of both pterygia in double-headed pathology with conjunctival autograft often is not possible. Several techniques have been proposed in the literature to address this surgical challenge. In 11 eyes, solomonetal.4 used amniotic membrane transplantation in double-headed pterygia and cited a recurrence rate of 9% (1/11 cases). The authors reported motility restriction and symblepharon formation evident in 3 patients, associated with the extensive removal of subconjunctival tissue and local injections of corticosteroid.

Further more, amniotic membrane may not be readily available at all institutions and is costly. Avisar et. al.6 described the irexperience in 10 eyes with the use of intraoperative mitomycin C after bare sclera excision of primary double-headed pterygia. No recurrences were noted with this technique, and only 1 patient experienced development of a pyogenic granuloma postoperatively. Concerns with the use of mitomycin C must still be considered, such as scleral melt, corneal perforation, iritis and glaucoma.13,14

Split-conjunctival graft technique has also been used to manage double-headed pterygia.5 In their series of 7 eyes, no recurrences were noted at a mean follow-up of 17 months. With this technique, sufficient superior conjunctival tissue and exposure is not always possible given that both grafts are harvested from the superior quadrant. Wuetal.7 devised a new
technique by using a conjunctival rotational autograft in combination with a conventional conjunctival autograft after pterygium surgery. In their series, the recurrence rate was 35% (7/20 cases), and the main postoperative complication was persistent injection over the conjunctival rotational autograft in 45% of cases (9/20 cases).

The recurrence rates after double-headed pterygium excision is reported to be 0% to 35%, depending on the chosen surgical procedure and size of the series published. Our recurrence rate of 5.56% is low and is consistent with reported recurrence rates after excision with conjunctival autograft for primary single-headed pterygia.

However, the low incidence rate (2.5%) would make recruitment a challenge, as noted in our series (0.6%). The minimum follow-up of 5 months was chosen because previous publications demonstrated that most recurrences occurred within 4 to 5 months. A longer follow-up period would be preferable nonetheless.

Strength of our study- Prospective, single surgeon, one sitting procedure, large sample size, conservation of conjunctiva.

Limitation of our study- Non-comparative, short follow up

Further randomised controlled trials to compare different methods are warranted to determined the optimal technique for the surgical management of double head pterygium, however recruitment of cases may be difficult because it was reported that the incidence of double head pterygium is less than 2.5%.

In summary, we describe a novel technique for the surgical treatment of double head pterygium, combined insitu conujunctivoplasty and conjunctival autograft.

The authors have no proprietary or commercial interest in any materials discussed in this article.

REFERENCES


20 Years Experience in Managing Non-Tuberculous Mycobacterial Keratitis at a Tertiary Care Center

Dr. Rashmi Mittal, Dr. Prashant Garg, Dr. Savitri Sharma

Mycobacteria other than M.tuberculous complex mainly exist in the environment as saprophytes and have been recognized as a cause of human disease for a very long time. Although the diseases caused by these organisms are uncommon compared with tuberculosis but there are enough reports of these organisms causing pulmonary and non-pulmonary infections.

Nontuberculous mycobacteria (NTM) are also known to cause a variety of ocular infections, including the infections of the cornea. In 1965 Turner and Stinson described the first case of NTM keratitis. Subsequently several reports of keratitis caused by the NTM have been published in the literature. Most of these infections are caused by two species i.e. M.fortuitum and M.Chelonei. The other NTM that have been reported to cause corneal infections include M.sulzi, M.flavescens, M.avium-intracellulare, M.gordonae and M.marinum.

Trauma and the metallic corneal foreign body in particular is the most common predisposing factor for NTM keratitis but it has also been reported following a variety of surgical procedures such as radial keratotomy, pterygium removal, cataract extraction, contact lens use, refractive surgery and keratoplasty. All the reports also highlight that non tuberculosis mycobacterial keratitis remains a challenge in terms of diagnosis and management. There is usually a delay in diagnosis and many cases do not respond to medical therapy and require surgical intervention.

In this manuscript we present our experience with large number of cases of corneal infections caused by this group of organisms.

MATERIALS AND METHODS

The study was approved (Ref. No. LEC 08045) by the Institutional Review Board of L V Prasad Eye Institute, Hyderabad, India. This is a retrospective case series. To identify the cases we reviewed the microbiology records of all cases of keratitis managed at L V Prasad Eye Institute Hyderabad, India, between January 1995 and April 2014. All the cases that showed a significant growth of NTM on culture were included in the series. According to the institute protocol all cases had a detailed history and slit lamp biomicroscopic examination. All subjects then were subjected to corneal scraping using sterile no. 15 surgical blade on Bard parker handle under 4% liganocaine topical anesthesia. The material obtained on scrapings was
smeared on clean presterilized glass slides for microscopic examination using Gram stain, Giemsa stain, and potassium hydroxide with calcofluor white preparation. 1% and 20% Acid fast stains (Kinyoun’s stain, Ziehl Neelsen stain) were used when there was a strong clinical suspicion of Nocardia or atypical mycobacteria infection. In addition, if Gram stain revealed gram positive bacilli (faintly stained) or unstained bacilli the smear was decolorized with acetone and restained by Ziehl Neelsen stain.

Scraped material was also inoculated on to sheep blood agar, chocolate agar, Sabourauds Dextrose agar, Potato Dextrose agar, Thioglycollate broth, Brain heart infusion broth and Non nutrient agar with E.coli overlay for Acanthamoeba. All media except Sabourauds Dextrose agar, Potato Dextrose agar were incubated at 37°C for a period of 7 days. Sabourauds Dextrose agar and Potato Dextrose agar were incubated at 27°C for a period of 7 days, and declared culture negative if there was no growth of bacteria, fungus, Acanthamoeba. The culture was considered positive or significant when there was (1) growth of the same organism on two or more media, or (2) confluent growth at the site of inoculation on one solid media, or (3) growth in one medium with consistent direct microscopy findings. NTM was identified by the colony morphology, rate of growth within 4 days and ability to grow on blood agar. If the colony on blood agar showed poorly stained gram positive bacilli, a Ziehl-Neelsen stain was done to confirm whether the organism was acid fast or not. M.Chelonei and M.fortuitum were distinguished from one another by Nitrate reductase test. In vitro antibiotic sensitivity was determined by Kirby-Bauer disk diffusion method.

The initial treatment was started based on smear results. All patients where smear examination did not reveal fungi or parasite received treatment with broad spectrum antibiotics. This was a combination of cefazolin 5% and aminoglycoside 1.4% till 1998, topical fluoroquinolone till 2002 and combination of cefazolin 5% with ciprofloxacin 0.3% after that in most cases. Once acid fast bacilli were identified either on smear or culture the treatment was modified to amikacin 2.5% or ciprofloxacin 0.3%. Some patients

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total number of isolates Tested</th>
<th>Sensitive</th>
<th>Sensitivity Pattern</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>48</td>
<td>45</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>Cefazolin</td>
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<td>1</td>
<td>–</td>
<td>49</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>24</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>49</td>
<td>7</td>
<td>–</td>
<td>42</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>28</td>
<td>6</td>
<td>–</td>
<td>22</td>
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<td>Gentamicin</td>
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<td>6</td>
</tr>
<tr>
<td>Oflaxacin</td>
<td>33</td>
<td>2</td>
<td>–</td>
<td>31</td>
</tr>
</tbody>
</table>
examined after 2005 received moxifloxacin 0.5% as primary therapy either alone or in combination of amikacin. All patients were closely followed up. If there was no response to medical therapy or in patients presenting with sever keratitis lamellar keratectomy was performed. Penetrating keratoplasty was done in all patients showing worsening on medical therapy.

RESULTS

Demographic profile

Out of 7950 culture positive bacterial keratitis cases, 52 showed a significant growth of nontuberculous mycobacteria, suggesting a prevalence of 0.65% for NTM keratitis, as shown in Figure 1. The patients varied in age from 13 to 74 years (Mean age 35.74 ± 13.33 years). 40 of these were males (77%) and 12 (23%) were female subjects. The duration of symptoms ranged from 2 days to 8 months (median 30 days) as shown in Figure 2. Trauma was the most common predisposing factor for NTM keratitis as depicted in figure 4.

Clinical presentation

Sixteen (30%) patients presented with visual acuity 20/200 or better. Twenty six (50%) patients had paracentral or peripheral infiltrate while remaining patients presented with central infiltrate. The infiltrate size varied from pinhead size to nearly 6 x 6 mm. The modes of presentation (Figure 3) of these ulcers could be classified as follows:

1. Deep stromal infiltrates– 30.8%
2. Ulcerative keratitis– 40.4%
3. Severe corneal thinning / perforation - 11.5%
4. Interface opacification/haze – 9.6%
5. Masquerading as fungal or viral keratitis - 17.3%

In none of the patients was the characteristic cracked windshield appearance documented. History of waxing and waning course, corneal infiltrate within surgical wound and presence of scar at the edge of active infiltrate were the features that helped us suspect infection by atypical mycobacteria in 40% of these cases.

Microbiology

Out of 52 cases Gram stain of the corneal scraping showed faintly stained Gram positive bacilli/unstained bacilli in 24 (46%) cases, Gram positive cocci in 2 cases (4%) and no organism was reported in 26 (50%) cases. Gram smears of 17/24 patients that showed faintly stained Gram positive bacilli/unstained bacilli were decolorized with acetone and re-stain by Ziehl Neelsen method. All the 17 smears showed acid fast bacilli.
The culture revealed concomitant growth of alpha hemolytic streptococci in one patient, Streptococcus pneumoniae in one patient and Staphylococcus epidermidis in two patients. The atypical mycobacterial growth was identified as M.Chelonei in 41 out of 52 patients (79%) and M.fortuitum in remaining 11 cases (21%). The results of in-vitro antimicrobial sensitivity test are shown in table 1. Majority of isolates (93.75%) showed sensitivity to amikacin and 87.5% of the isolates were sensitive to gentamycin. Only 47% isolates were sensitive to first generation fluoroquinolone ciprofloxacin.

**Treatment outcome**

The principle drug used in this series following culture and sensitivity results was topical Amikacin 2.5% either alone or in combination with fortified Cefazolin or Ciprofloxacin.
In 35 cases (67.31%) patients the infection resolved to the medical management either alone or combination with superficial keratectomy. These included three patients that had infection after LASIK and had undergone amputation of the LASIK flap and the patient with infection of cataract surgical wound that was subjected to patch graft. 6 patients (11.54%) required penetrating keratoplasty and one patient (1.9%) was advised evisceration as he presented with a large infiltrate associated with perforation. However the patient did not agree for the surgery. 10 (19.23%) patients were lost to follow-up.

**DISCUSSION**

Nontuberculous mycobacteria are aerobic, non-sporulating, non-motile bacilli. Because of many differences in characteristics from Mycobacterium tuberculosis these are labeled as “atypical”. These are relatively uncommon cause of infection of the cornea. In a large sample of keratitis cases seen at LV Prasad Eye Institute, atypical mycobacteria were isolated from less than 1% of culture positive bacterial keratitis cases. Literature search using PubMed and review of literature in English language clearly showed that most of the reports in literature are either single case reports or small case series. We could find only two large case series with 24 cases in one and 22 cases in another series. The present series with 52 cases is therefore going to be a useful addition to the literature toward understanding various aspects of this rare disease.

Some of the common characteristics of the keratitis caused by the NTM as seen in this series and reported previously are; delayed diagnosis, need for prolonged medical treatment, difficulty in controlling infection with medical treatment alone and need for surgical intervention.

Delayed diagnosis: Since these organisms are relatively uncommon isolates from keratitis cases these are often not considered in differential diagnosis. The clinical features in many of these cases mimic herpes simplex viral keratitis, Acanthamoeba keratitis or fungal keratitis (17.3% in this series). The diagnosis is also missed because many cases present with deep stromal infiltrates making it difficult to obtain an adequate sample for microbiology studies (30.8% in this series). Further, a standard microbiology protocol does not include acid fast staining.

A high degree of clinical suspicion will help an early diagnosis. The NTM must be suspected in all cases of keratitis presenting with 1) history of corneal foreign body, or ocular surgery, 2) a slowly progressive disease with localized infiltrate, and 3) waxing and waning clinical course. In the present series relatively prolonged duration of symptoms associated with localized infiltrate, history of waxing and waning course of keratitis, presence of scar
at the edge of active infiltrate and corneal infiltrate within surgical wound helped us suspect infection by the atypical mycobacteria in 40% cases. In all such cases we must include an additional smear for acid fast staining. Further, the presence of partially stained or unstained bacilli on microscopic examination of smears (46% in this series) can be another important clue to suspect infection by atypical mycobacteria. Such smears can be decolorized and counter stained with modified Ziehl Neelsen stain to demonstrate acid fast bacilli. Although, Ford et. al. recommended use of special media such as Lowenstein Jensen for isolation of these organisms our experience suggest that most of these organisms grow on routine culture media within one week. However, it is possible that we have missed cases caused by the slow growers and the organisms that have more fastidious growth requirements.

Runyon divided NTM in to 4 groups based on cultural characteristics and the development of pigmentation when exposed to light. Group 4 NTM are also called as rapid growers and the most important members of this group include M.Chelonei and M.fortuitum. Most reported cases of NTM keratitis have been caused by M.Chelonei and M.fortuitum. In our study we found M.Chelonei (79%) was the most common causative species of NTM keratitis followed by M.fortuitum (21%). Since the identification of rapidly growing mycobacteria by using biochemical tests sometimes fail to discriminate between closely related species such as M.abscessus and M.chelonea the speciation must be achieved by molecular methods, preferentially sequencing of rpoB and hsp65 genes. The species identification in this series was based on biochemical tests. Accurate speciation will help us better understand epidemiology, clinical features, antibiotic susceptibility and treatment outcomes of this rare infection. The use of biochemical methods for species identification in this study, we feel, is a major limitation.

The second important aspect of this infection is medical treatment. In-vitro antibiotic sensitivity results from various studies uniformly suggest amikacin to be the drug of choice against infections by NTM. In our series 93.75% isolates were sensitivity to amikacin and 87.5% were sensitive to gentamicin. Only 47% NTM isolates in this series were sensitive to ciprofloxacin. For few isolates we included gatifloxacin for in-vitro antibiotic susceptibility test and found relatively poor susceptibility.

Based on the in vitro antimicrobial susceptibility results, we used amikacin as the drug of choice for most of our cases. In 35 (67.31%) patients the infection resolved with medical management either alone or combination with a limited surgical procedure such as flap amputation or superficial keratectomy. Although the anecdotal experience with topical ciprofloxacin has been limited and the clinical impression of its effectiveness has been mixed (26-27) we did not find good response with this antibiotic.
Another important aspect of this infection is the need for prolonged medical treatment and waxing and waning course even on treatment with susceptible antibiotic. Treating physicians must be aware of this characteristic. Such exacerbations after prolonged remission should not be considered inflammatory phenomenon and physicians must abstain from using topical corticosteroids.

Since nearly 30% cases of corneal infection by NTM do not respond to current medical treatment surgery plays a very important role in the management of this disorder. Surgery can be in the form of lamellar keratectomy or full thickness penetrating keratoplasty. Five patients in this series were subjected to lamellar keratectomy, one to patch graft and 6 patients required full thickness penetrating keratoplasty. The experience of the authors of the two other large series had been similar.\textsuperscript{2,18}

**CONCLUSION**

This is the largest series of corneal infection by NTM and is a useful addition to the literature helping us understand various aspects of this disease. This series further highlights difficulties in diagnosis and management of this disorder. A high degree of clinical suspicion and careful microbiology will help early diagnosis. Although there are reports of use of fluoroquinolones, topical amikacin 2.5% is the drug of choice in the management of this condition. In spite of high in-vitro susceptibility the medical treatment may fail to control infection in a larger number of patients.

**REFERENCES**


