Comparative evaluation of Amniotic Membrane Transplantation with conventional medical treatment versus conventional medical treatment alone in Suppurative Keratitis

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Citation

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Abstract

Background : To study the role of amniotic membrane transplantation in infective keratitis.
Methods : A prospective, randomized study on two groups of 20 patients with infective keratitis was done. Group A : only conventional medical treatment. Group B: conventional medical treatment with amniotic membrane transplantation was done.

Results The mean age of patients was 46.30 ± 19.23 years. At 1 week follow-up, the improvement in symptoms like pain, redness, photophobia, watering and discharge was present in 24%, 4%, 25%, 28% respectively in patients without AMT, whereas it was 48%, 26%, 55% and 48% respectively in patients with AMT. The improvement in signs like size of corneal ulcer and hypopyon was 10% and 13% in patients without AMT, whereas it was 35.2% and 54% respectively in patients with AMT.

Conclusion : Amniotic membrane transplantation with conventional medical treatment in infective keratitis is more effective than medical treatment alone.

Background: Infective keratitis caused by bacteria, fungi, viruses or parasites leads to permanent corneal opacity or persistent epithelial defect [1,2,3] which is potentially sight threatening ocular problem. The cornea being avascular, is particularly susceptible to infection and many patients have poor clinical outcome, if aggressive and appropriate therapy is not promptly initiated [1,2] .

The primary objective of therapy for infectious keratitis is to eliminate infective organism, prevent tissue destruction (stromal keratolysis) and corneal structural alterations. Traditionally, the most effective treatment of infective keratitis is obtained by topical delivery of antimicrobials directly to the infected cornea [1] . Supplementation with systemic antimicrobial is required in some cases [8] .

However, in cases of progressive ulceration, surgical intervention becomes a necessity [1] . The ophthalmic literature describes a multitude of surgical procedures for corneal reconstruction. Recently, preserved human amniotic membrane has emerged as a useful tool in the reconstruction of the damaged ocular surfaces [4,5] . Certain characteristics like anti-inflammatory, antiangiogenic, antiinfective, antifibroblastic activities make the amniotic membrane ideally suited to its application in ocular surface [4,5] .

In 1995, Kim and Tseng [6] introduced the modern way of using amniotic membrane in its current form because of its action in facilitating epithelization and reducing stromal inflammation and scarring.

All the characteristics of amniotic membrane e.g. anti-inflammatory, antiangiogenic, antiinfective and antifibroblastic are useful in corneal ulcer healing. Only few studies are available in which amniotic membrane transplantation was done for infectious corneal or corneoscleral ulcers [7,8] . We conducted this study to compare the efficacy of amniotic membrane transplantation in

addition to conventional therapy vs conventional therapy alone in patients with infective keratitis.

MATERIALS & METHODS

This prospective, randomized study was conducted on 40 patients having infective keratitis who were randomly divided into two groups of 20 each. A written informed consent was taken from all the patients as per guidelines of Helsinki Declaration.

Patients of group A received only conventional medical treatment while patients of group B received conventional medical treatment along with underwent preserved human amniotic membrane transplantation.

Patients with typical viral ulcers on clinical evaluation, ulcer associated with autoimmune condition, atheromatous corneal ulcer, perforating corneal ulcer having size of more than 2mm and corneal ulcer with endophthalmitis were excluded from the study.

Detailed history was taken followed by ocular examination in all the patients. Symptoms like pain, redness, photophobia, watering & discharge were recorded and graded.

Grades of pain : Grade 0 ; no pain , Grade 1; occasional mild pain,Grade 2; constant mild pain, Grade 3; moderate to severe pain,Grade 4 ; constant severe pain.

Grades of redness : Grade 0 ; no redness, Grade 1; redness in one quadrant ,Grade 2; redness in two quadrants,Grade 3; redness in four quadrants,Grade 4; redness all around

Grades of photophobia : Grade 0; no photophobia, Grade 1 ; photophobia only in bright light,Grade 2; photophobia in day light, Grade 3; photophobia in dim light, Grade 4; photophobia without light.

Grades of watering and discharge : Grade 0 ; no watering or discharge,Grade 1; only watering ,Grade 2; discharge occasionally,Grade 3; constant discharge,Grade 4 ; foul smelling constant discharge.

Ocular examination included visual acuity testing on Snellen's chart and thorough slit lamp examination of conjunctiva, cornea, anterior chamber, iris, pupil, and lens. Conjunctival congestion, hypopyon size, ulcer size, corneal infilterates were all graded.

Grades of conjunctival congestion : Grade 0; normal, Grade 1; one quadrant congested , Grade 2 ; two quadrant congested,Grade 3; three quadrants congested,,Grade 4 ; congestion all around.

grades of corneal ulcer size: grade 0; less than 0.5 mm, grade1; 0.5–1mm, grade 2; >1 – 2 mm,grade 3; > 2 – 5 mm,grade 4;> 5 mm.

Grades of corneal infilterates : Grade 0; no infilterates, Grade 1; only upto epithelial surface,Grade 2; infilterates may be dense but superficial and limited to ulcer base, Grade 3; dense infilterates extending to mid stroma,Grade 4; dense infilterates extending deeper than mid stroma or upto sclera .

Grades of hypopyon :Grade 0; no hypopyon, Grade 1; upto 1mm, Grade 2; >1-2mm, Grade 3; >2-3 mm, Grade 4;> 3 mm.

Grades of visual acuity : Grade 0 ; Normal, Grade1; 6/6-6/18, Grade 2; 6/24-6/60 Grade 3; < 6/60-3/60,Grade 4;< 3/60.

Associated ocular signs like blepharitis, dacryocystitis, dry eyes, and corneal sensations were also noted. Posterior segment was evaluated by direct and indirect ophthalmoscopy or by USG when the media was hazy.

Clinical diagnosis of bacterial or fungal ulcer was made by history and ocular examination. Microbiological examination included Gram Stain and KOH wet mount of corneal scrapings followed by culture for bacteria or fungi. In patients with clinical diagnosis of bacterial corneal ulcer and negative KOH smear for fungus, fortified antibiotics drops (cefazolin 5% half hourly, amikacin 5% half hourly) were started and ancillary treatment (atropine eye drop, antiglaucoma eye drop or oral acetazolamide 500mg qid, oral vitamin C qid) was also added. With clinical diagnosis of fungal corneal ulcer with or without KOH positive report for fungus, patients were started on antifungal eye drops (natamycin 5% one hourly). In severe corneal ulcer with KOH positive for fungus, patients were also started on oral antifungal drugs. (Tab. Itraconazole 100mg bid)

AMNIOTIC MEMBRANE – PREPARATION AND STORAGE

Amniotic membrane (AM) preparation & storage [59] was done as mentioned below:

Detailed medical history and clinical condition of potential donor was judged to exclude risk of tissue transmissible infections.

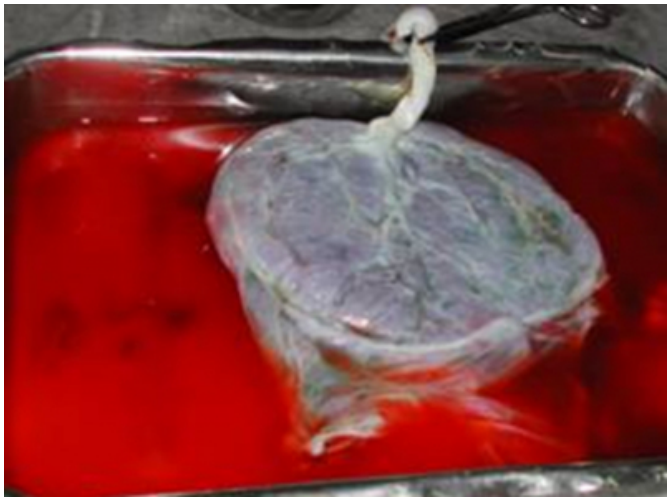
Consent of Donor for donation of placenta & subsequent use.

Donors screened for HIV type 1 & 2, Hepatitis B & C virus, Syphilis.

Amniotic membrane procured from elective caesarian section (Fig 1) routinely done for their respective indications. No caesarian delivery was done for obtaining amniotic membrane only.

Figure 1

Figure 1 : Fresh placenta in Eagles' minimum essential medium



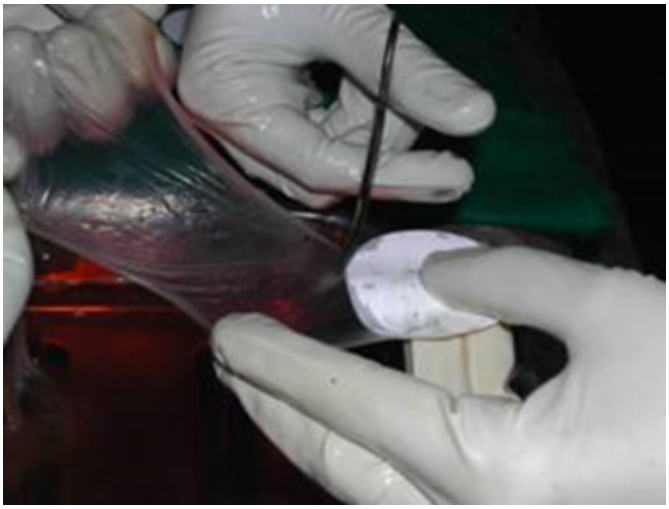
In the operation theatre, the amniotic membrane dissected from placenta in two larger bits. As much of chorion as possible was peeled out before the bits were dropped into sterile bottle containing 50 ml of transport medium. The transport media (Eagles' minimum essential medium) (EMEM) supplemented with 3.3% L-glutamine and antibiotics (50 g/ml gentamicin, 100 units/ml penicillin, 200 g/ml ciprofloxacin and 1mg/ml amphotericin B).

The placenta was first washed free of blood clots with EMEM containing antibiotics. The inner AM was then separated from chorion by blunt dissection.

Amniotic membrane was spread uniformly on individually sterilized 0.22 m nitrocellulose membrane with the basement membrane side (Fig 2).

Figure 2

Figure 2 : Amniotic membrane being cut and spread on sterilized 0.22 m nitrocellulose membrane.



The membrane was cut into 4x 4 cm pieces and was placed in the preservative medium in 50 ml wide mouthed bottles. The preservative medium used was 1:1 (vol/vol) ratio of sterile glycerol and EMEM with 3.3% L- glutamine and antibiotics (25 g/ml gentamicin, 50units/ml penicillin, 100 g/ml ciprofloxacin and 0.5 mg/ ml amphotericin B).

The amniotic membrane was stored at -80 C.

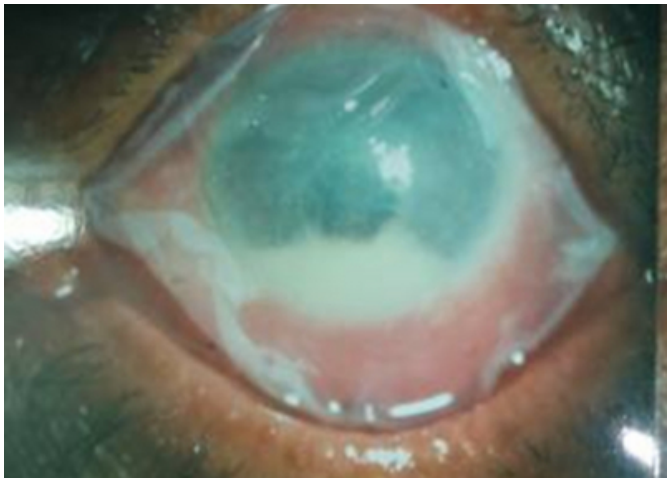
Technique of Amniotic Membrane Transplantation:

Amniotic membrane transplantation (AMT) was done after taking informed consent from the patient. The procedure was done under topical or peribulbar anaesthesia. Under all aseptic conditions, the base of ulcer and surrounding cornea was cleaned of necrotic tissue. After thawing, amniotic membrane was removed from the filter paper and spread over the cornea with the basement membrane side facing up. The side of the basement membrane was distinguished from the stromal side by touching it with sponge; the later being sticky, but not the former. The amniotic membrane was trimmed to cover the entire cornea extending beyond the limbus all around by 3mm (Fig 3). It was sutured to the bulbar conjunctiva using 10-0 nylon suture. After AM transplantation, patients were continued on same medical treatment.

Postoperatively, topical and systemic antibiotics were continued as required. Patients were followed up till 3 months. At each follow up, detailed ocular examination was

Figure 3

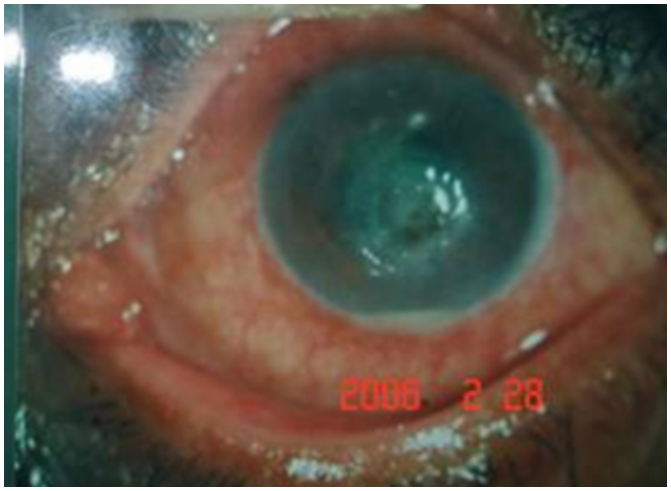
Figure 3 : Amniotic membrane transplanted on suppurative fungal corneal ulcer



carried out and symptoms (pain, redness, photophobia, watering& discharge) and signs (conjunctival congestion, ulcer size, infilterate size, hypopyon size, final visual acuity) were scored and graded and compared at different time periods (Fig 4).

Figure 4

Figure 4 : Postoperative condition of same patient after 3 weeks showing markedly decreased corneal infilterates and hypopyon.



In statistical analysis for inter group comparison 'Mann Whitneys' test was used and for intra group comparison 'Wilcoxon Sign Rank' test was used.

RESULTS

In this study, age of the patients ranged from 15-84 years with 37.5% between 41 to 60 years of age. Mean age in Group A was 41.85 ± 17.42 years while in Group B it was 50.75 ± 20.34 years.

There was male preponderance with 82.5% (n=33) of patients being males. There were 85% (n=17) males in Group A as compared to 80% (n=16) in Group-B.

In the analysis of predisposing factors, history of injury was present in 40% (n=8) patients in Group A and 55% (n=11) in Group B. The most common mode (30%) of injury was with organic matter. The other modes of injury were with metallic objects (18%) and insect bite (3%).

Grades of corneal ulcers:

Clinical diagnosis was made after complete history and ocular examination and corneal ulcers were graded. Overall, 52.5% (n=21) patients had moderate grade corneal ulcers. In Group A, 60% (n=12) patients had moderate grade corneal ulcers, 35% (n= 7) had severe grade and 5% (n=1) of patients had mild corneal ulcer. In Group B, 55%, (n= 11) had severe grade while 45%,(n = 9) had moderate grade of corneal ulcer.

Types of corneal ulcers:

Fungal corneal ulcers were the commonest, present in 62.5% of patients followed by bacterial (22.5%) and mixed ulcers (15%) (Table 1). Out of total 25 fungal corneal ulcers, 60% (n=15) smears were positive for KOH wet mount and 32% (n=8) samples were positive for culture. In Group A, 58.33% smears were positive for KOH wet mount, while culture positivity was seen in 25% samples. In Group B, 61.5% smears were positive for KOH wet mount and 38.5% samples were culture positive (Table 2) .

Fungal culture showed growth of Aspergillus flavus in 1 (8.3 %), Fusarium oxysporium in 2 (16.6 %) patients in group A. While in group B the organisms identified were Fusarium oxysporium in 1 (7.7%), Aspergillus flavus in 3 (23.1%) and Drechslera species in 1 (7.7%) patient.

In cases of bacterial corneal ulcers, none showed positive gram staining. All the bacterial ulcers were negative for KOH wet mount, Gram staining and culture. Out of 6 mixed corneal ulcers, 1 from Group A and 2 from Group B were positive for KOH wet mount and only 1 mixed ulcer of Group A showed growth of E.Coli in culture.

During follow up of patients, in Group A, two patients with severe grade ulcers underwent therapeutic keratoplasty in 3 rd and 5 th week respectively and in Group B, three patients

with severe grade ulcers underwent therapeutic keratoplasty in 3rd week. The patients who underwent therapeutic keratoplasty were not evaluated further in the study. Hence in Group A there were 19 patients for the follow up after 1 month and 18 patients for follow up after 3 months, whereas in Group B there were only 17 patients left for follow-up after 1 month.

Comparison of various signs and symptoms at different time intervals in two groups was as followed.

Pain: At presentation 8 (40%) patients in group A and 11 (55%) patients in group B had moderate to severe pain (Grade III). After 1 week, grade III pain was still present in 7 (35%) patients in group A as compared to 2 (10%) patients in groupB ; the improvement in pain in Group B was statistically significant (p value < .05). At subsequent visits, the difference was not significant .

Redness : 60% patients in group A and 80% patients in group B had redness all around (grade IV) at presentation which decreased to 50% and 45% patients respectively at 1 week. After 1 week, none of the patients in group A got relieved of redness, while in group B, 3 patients got relieved of it. After 1 week, the improvement in redness was more in group B (p value <0.05).

Photophobia : 35% patients in group A and 60% in group B had grade III photophobia at presentation which decreased to 15% and 5% patients respectively after 1 week. The improvement in photophobia was more in group B (p value < .05).

Watering and Discharge (W & D) : At presentation, 65% patients in group A and 45% in group B had grade III W & D. After 1 week, grade III W & D was still present in 5 (25%) patients in group A where as in group B only 1 patient (5%) was having grade III W & D. After 1 week, the improvement in W & D was more in group B which was statistically significant (p value < .05).

Conjunctival Congestion At presentation, 95% patients in group A and all the patients in group B had grade IV (congestion all around) conjunctival congestion. After 1 week, grade IV conjunctival congestion was still present in 85% patients in group A and 80% patients in group B and this difference in two groups was not statistically significant.

Corneal ulcer size: At presentation, 50% patients in group A and 45% patients in group B had grade III size of corneal ulcer, while grade IV (> 5 mm) size of corneal ulcers were present in 30% patients of group A and 55% patients of group B. After 1 week, grade III ulcer was present in 35% patients in group A and 25% patients in group B, where as grade IV size of ulcers were present in 25% patients of group A and 20% patients of group B. The improvement in ulcer size was more in group B as compared to group A after 1 week and 3 months. (p value <0 .05).

Corneal infiltrates: At presentation, 50% patients in group A and 35% patients in group B had grade III corneal infilterates while 35% patients in group A and 40% patients in group B had grade IV corneal infiltrates. After 1 week, grade III corneal infiltrates were present in 30% patients in group A and 40% patients in group B whereas grade IV corneal infiltrate were present in 25% patients of group A and 10% patients of group B. The change in size of infilterates between the two groups was not statistically significant.

Hypopyon : At presentation, 5% patients in group A had grade IV hypopyon, where as in group B, 30% patients had grade IV hypopyon. After 1 week, there was no improvement in hypopyon in group A while grade IV hypopyon remained only in 10% of patients of group B. This improvement was statistically significant (p value <0 .05).

Visual Acuity: At presentation, 65% patient in group A and 80% in group B had grade IV visual acuity. After 3 months of follow up, 10% patients from each group gained normal vision (grade O). Grade I visual acuity was present in 40% patients of group A and 10% patients of group B. Grade IV visual acuity was still present in 10% patients of group A and 20% patients of group B at 3 months of follow up.This improvement in visual acuity was statistically significant (p<0.05).

DISCUSSION

Infective keratitis is a sight–threatening problem. Infectious organisms with infilterating inflammatory cells lead to production of various enzymes in presence of injured corneal epithelium leading to stromal keratolysis. Limbal vessels ingrowth may create an additional source for polymorphonuclear neutrophils. It requires an aggressive approach for management of corneal ulcer and thereby saving the vision threatening complications. Amniotic membrane has been found to be a useful tool in the reconstruction of the damaged ocular surface [4,5] . Amniotic membrane consists of a thick basement membrane and an

avascular stroma [9] that expresses mRNAs for number of growth factors and contains several growth factor proteins that benefit epithelisation [10,11] . Amniotic membrane precludes polymorphonuclear cells infiltration and decreases lipid peroxidation and keratocyte death [12] . It decreases stromal inflammation and has suppressive effect on the expression of two most potent pro-inflammatory cytokines [13] , interleukin-1 and interleukin-1 [14] . It also accelerates apoptosis of polymorphonuclear neutrophils and prevents resolution [5,12] of ocular surface collagen. Hao et al. identified mRNA for cytokines IL1 receptor antagonist and IL10 in amniotic membrane epithelium. These cytokines are potent inhibitors of inflammation [15] . All these effects, directly or indirectly modulate the inflammatory process induced by infection leading to beneficial effects in infectious keratitis.

Only few studies are there, in which amniotic membrane transplantation was done for infections involving cornea and sclera with encouraging results [7,16] . Infective keratitis mainly affects young adults and older population who are outdoor workers, especially working in the fields. In this study, age ranged from 15 to 84 years with mean age of 46.30 ± 19.23 years. 37.5% of the patients were between 41 to 60 years and 32.5% were in the age group of 21-40 years. This is similar to a study from South India [18] in which most of the patients (43.5%) were of the age group of 41 to 60 years. Bimodality in the patient's age group can be attributed to the fact that young adults are more physically active and at a higher risk for corneal injury, and older population might have predisposing ocular surface or eye lid diseases.

Infective keratitis is more common in males as is evident by various studies. Basak et al. [17] in their study reported 70.6% males and 29.4% females. In another study by Srinivasan et al. [18] , 61.3% were males and 38.7% were females. In our study also there were 33 (82.5%) males and only 7(27.5%) females. Males get corneal infection more commonly because they usually work in the fields where chances of corneal trauma are more.

Predisposing risk factors for microbial keratitis vary tremendously with geographical location [1,14] . Non-surgical trauma to the eye accounted for 48.6–65.4% of all corneal ulcers in the developing countries like Nepal [19] and India [18] . In the United States it is contact lens wear that is a major risk factor for microbial keratitis [14] . In our study, history of injury to the cornea was present in 19 (47.5%) patients. The most common mode of injury (30%) was organic matter. Basak et al. [17] found history of injury to cornea in 82.9% of patients, with vegetative matter being the most common mode of injury, present in 59.6% of patients. In another study by Srinivasan et al. [18] injury to the cornea was present in 65.4% patients, with injury from paddy (25.4%) being the most common followed by vegetative matter (15.1%).

In our study, fungal corneal ulcers were the commonest amongst all types of ulcers. Overall fungal corneal ulcers were present in 26 (65%) patients followed by bacterial and mixed ulcers in 8 (20%) and 6 (15%) patients respectively which is similar to study reported by Basak et al [17] in which 59.3% patients were having fungal infection. In another study by Leck et al [20] fungi were identified as the principal aetiological agents causing corneal ulceration in 44% of all cases in Ghana.

Role of microbiological evaluation is useful in making the diagnosis of infective keratitis. Smear and culture positivity varies depending upon amount of material available, method of corneal scraping, availability of fresh culture plates, and a rapid transport system [1,21] . In our study, out of 25 fungal corneal ulcer , 60% (15 patients) smears were positive for KOH wet mount and 32% (8 patients) samples were positive for culture. The organisms isolated were Aspergillus flavus in 4 (16%), Fusarium oxysporium in 3(12%) and Drechslera species in 1 (4%) patient. In a study by Basak et al. the most common fungal pathogens were Aspergillus species [17] . Leck et al. also reported Aspergillus and Fusarium species as the most frequently fungal pathogens isolated from cases of fungal keratitis [20] .

In our study, all the clinically appearing bacterial ulcers (9 patients) were negative for KOH wet mount, Gram staining and culture. 45% patients were negative for both microscopy and culture. In a study by Leck et al., it was not possible to determine the aetiological agent in 50% of corneal ulcer cases in Ghana [20] . The lower rate of isolation might be attributed to the use of topical antibiotics before taking samples. In our study majority of the cases were referred patients and had taken some or the other treatment before presenting to us.

Severity of the ulcer like larger size, dense infilterates and older age has been found to be associated with bad prognosis for infective keratitis. Miedziak et al. in their study had concluded that older age, delay in referral to cornea specialist and large size of ulcer are risk factors for poor outcome of microbial keratitis [22] . 5 (12.5%) patients in our

study required therapeutic keratoplasty during the course of study. All these were large ulcers with dense infilterats. Three out of these five patients (60%) were in the age group of 60 years or above and all of these patients presented to us after two weeks of infection. Thus our study agrees with earlier observations that older age, delay in presentation, larger size and dense infilterats are bad prognostic signs.

In various studies, amniotic membrane has been found to provide symptomatic relief in various forms of ocular surface disorders [4,5] . Pires et al. performed AMT in 50 consecutive eyes with symptomatic bullous keratopathy and found that 43(90%) of eyes became free of pain postoperatively. Epithelial defect healed rapidly in 45 out of 50 (90%) eyes within 3 weeks [23] . Heiligenhaus A et al. studied retrospective, non-comparative case series of seven patients with acute ulcerative and necrotising herpetic stromal keratitis. Single or multilayer AMT with epithelial side facing up was performed. The main outcome measures were wound healing of the corneal ulcers and decrease of stromal inflammation [24] .

In our study, there was definite improvement in symptoms like pain, redness, photophobia, and watering and discharge with amniotic membrane transplantation as compared to patients not undergoing AMT.

With amniotic membrane transplantation, moderate to severe pain decreased from 11(55%) patients at presentation to 2(10%) patients after one week, whereas without amniotic membrane transplantation moderate to severe pain decreased from 8 (40%) patients on presentation to 7 (35%) patients after 1 week. Similarly redness also decreased after amniotic membrane transplantation. At presentation, 12 (60%) patients without AMT and 16 (80%) patients with AMT had redness all around. After 1 week redness all around was still present in 10 (50%) patients without AMT, but only in 9 (45%) patients with AMT. Other symptoms like photophobia with watering and discharge showed more improvement in patients undergoing amniotic membrane transplantation. At presentation, 7 (35%) patients without AMT and 12 (60%) patients with AMT had photophobia in dim light. After 1 week, photophobia in dim light was still present in 3 (15%) patients without AMT where as, amongst patients with AMT, only 1 (5%) patient was having photophobia in dim light. More number of patients (30%) got relieved of photophobia after amniotic membrane transplantation as compared to patients without AMT. Watering and discharge decreased from 9 (45%) patients at presentation to only 1 (5%) patient after 1week with AMT where as, watering and discharge was still present in 25% of patients without AMT.

The symptomatic relief might be because of mechanical or physical effect of amniotic membrane. Amniotic membrane acts as a biological bandage to cover inflammed areas, which not only favorably influences the healing process but also has a favorable effect on the level of pain and discomfort experienced by the patient [4,5] .

In our study, signs of infective corneal ulcers improved more in patients with amniotic membrane transplantation as compared to the control group. At presentation, 10 (50%) patients without AMT and 9 (45%) patients with AMT had ulcer size of 2 to 5 mm. Ulcer size more than 5 mm were present in 6 (30%) patients with AMT and 11(55%) patients without AMT. After 1 week ulcer size of 2 to 5 mm was present in 7 (35%) patients and ulcer size more than 5 mm in 5 (25%) patients without AMT. Where as, after 1 week ulcer size of 2 to 5 mm was present in 5 (25%) patients and ulcer size more than 5 mm in 4 (20%) patients with AMT. After 1 week and 3 months, the improvement in ulcer size was significantly more in patients with AMT as compared to patients without AMT.

In our study 1 (5%) patient without AMT and 6 (30%) patients with AMT were having hypopyon of more than 3 mm. After 1 week, hypopyon of more than 3 mm was still present in 1 (5%) of the patient without AMT while in patients with AMT, only 2 (10%) patients had hypopyon more than 3 mm. After 1 week, the improvement in hypopyon was significantly more in patients with amniotic membrane transplantation.

The results of this study indirectly indicate that the actions of anti-bacterial and anti-fungal drugs was not hampered by amniotic membrane. Topical drugs might have reached the cornea through the amniotic membrane itself or entered from the gap between the sutures of amniotic membrane. Kim et al [25] (2001) evaluated penetration and drug levels in tears after topical ofloxacin instillation in rabbit eyes with AMT. The mean tear levels of ofloxacin in AMT group were higher than those in non-AMT group. So he concluded that amniotic membrane has some potential to act as an effective drug delivery system.

Faster healing of ulcers with amniotic membrane transplantation might be because of anti-inflammatory, anti-angiogenic, anti-infective and anti-fibroblastic activity of

amniotic membrane [45] .

Kim et al. performed AMT in eyes with infectious corneal ulcer. The corneal surface healed successfully and recurrence of microbial infection was not noted in any case. They concluded that AMT could be useful adjunctive procedure for management of infectious corneal ulcer [11] . Ma DH et. al [7] studied the efficacy and safety of cryo-preserved human amniotic membrane graft as a patch graft to reduce stromal melting and promote re-epithelisation in extensive scleral and corneoscleral infectious ulcers. The infections were caused by Pseudomonas, fungi, and Atypical Mycobacterium. They concluded that amniotic membrane graft could be considered as an alternative biomaterial to improve wound healing in scleral and corneoscleral ulcerations [7] . In this regard, our study agrees with the observations of Kim et al. [11] and Ma DH et al. [7] .

The above discussion, compared with the results of our study clearly demonstrates that amniotic membrane transplantation is beneficial in infective corneal ulcers. Combined with conventional treatment, it can be used as a treatment modality in case of moderate ulcers, and as a temporary measure for symptomatic relief in severe ulcers.

Conclusions: This study showed encouraging results of amniotic membrane transplantation along with conventional treatment in infective keratitis in improving both symptoms and signs, but small size of sample was the limiting factor in our study. Hence large prospective and controlled trials with more number of patients are required for better assessment of role of amniotic membrane transplantation in addition to conventional medical treatment in patients with infective keratitis.

List of abbrebiations used :

AMT : Amniotic membrane transplantation

AM: Amniotic membrane

USG : Ultrasonography

EMEM : Eagles' minimum essential medium

Gr : grade

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