Original Research Article

Role of Amniotic Membrane Transplantation in Corneal Ulcers

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Abstract

Aim: To evaluate the efficacy of amniotic membrane transplantation in corneal ulcers and to observe the complications of amniotic membrane transplantation in corneal ulcers. *Material and methods:* Ours was a prospective cross- sectional study which was conducted over a period of 1 year at Regional Institute of Ophthalmology, PGIMS, Rohtak on 30 patients of microbial keratitis on whom amniotic membrane transplantation was done. Signs of ulcer healing- pain, congestion, size of ulcer, BCVA, anterior chamber depth and anterior chamber reaction were recorded on every subsequent follow up. *Result:* There was significant reduction in post-operative pain and congestion. Anterior chamber depth was not changed significantly. *Conclusion:* Amniotic membrane transplantation can be used as an adjuvant treatment in bacterial and fungal corneal ulcers for early healing, less vascularisation and scarring and better final visual outcome.

Keywords: Amniotic membrane, corneal ulcers, corneal vascularization, corneal scarring.

INTRODUCTION

Corneal ulcer is a challenging ocular condition which is very difficult to manage even after introduction of potent antibiotics and antifungals. Since it takes long time to heal it increases the total morbid period and decreases the productive period of life and affects the economy of developing countries like India. It usually ends in corneal opacity with or without vascularization decreasing the final visual acuity. If not managed properly it may lead to complications like glaucoma, corneal perforation and endophthalmitis. Also, vascularization makes the cornea vulnerable for graft rejection after penetrating keratoplasty. Hence, the ultimate aim of treating a corneal ulcer is quick healing with least scarring and vascularization. Now a days, the most emerging treatment for better outcome of corneal ulcer is amniotic membrane transplantation. The human amniotic membrane is the innermost layer of the placenta, which is composed of the outer chorion of maternal origin and the inner amnion of fetal origin. Histologically, the amniotic membrane is composed of three layers:

- **Epithelial monolayer:** It consists of a single layer of cuboidal cells arranged uniformly on the basement membrane with a large number of microvilli on the apical surface. These cells are nonviable even in fresh amniotic membrane. Current cryopreservation method for tissue storage destroys these cells by cell membrane disruption.

- **Thick basement membrane:** This is a compact layer composed of reticular fibers. The basement membrane of the amniotic membrane contains collagen types IV, V, VII as well as fibronectin and laminin which are also present in conjunctival and corneal basement membrane. Analysis of sub-chains of type IV collagen and laminin reveals that amniotic basement membrane closely resembles conjunctival more than corneal basement membrane [1]. It supports adhesion, growth and differentiation of epithelial basal progenitor cells as well as facilitates epithelial cell migration and prevents apoptosis of epithelial cells. The expression of pigment epithelium-derived growth factor can explain the antiangiogenic action of amniotic membrane [2]. This layer is resistant to cryopreservation.

- **Stromal matrix:** This layer is avascular, hypocellular and contains fibronectin, collagen type I, III, and V, glycosaminoglycans like hyaluronic acid, decorin, bi-glycan and non-glycosylated lumican 3]. It suppresses the expression of certain inflammatory cytokines like IL-1α, IL-1β, IL-8, interferon γ, tumor necrosis factor β, basic fibroblast growth factor and platelet derived growth factor [4]. It also attacks and

sequesters inflammatory cells infiltrating into the ocular surface and secretes natural inhibitors to various proteases [5]. Hyaluronic acid of stromal matrix suppresses TGF-β signaling, and myofibroblast differentiation of human corneal, limbal, conjunctival and pterygium fibroblasts [6, 7]. In addition amniotic membrane contains several neurotrophins [8] and nerve growth factor [9].

Amniotic Membrane helps in
1. Proliferation and differentiation of stromal fibroblasts.
2. Promoting epithelial cell migration, adhesion and differentiation.
3. Supporting the growth of the epithelial progenitor cells by prolonging their life span and maintaining their clonogenicity.
4. Suppressing the expression of certain inflammatory cytokines that originate from the ocular surface epithelium, including interleukin 1α (IL-1α), IL-1β, IL-2, IL-8, interferon γ, tumor necrosis factor-α and platelet derived growth factor.
5. Reducing vascularization and scarring.

Because of its strength, elasticity, semitransparency, healing property and resemblance with ocular tissue, amniotic membrane appears to be a suitable tissue for the management of corneal ulcers. Amniotic membrane transplantation has been performed for persistent corneal epithelial defects [10], neurotrophic corneal ulcers [11], leaking filtering blebs after glaucoma surgery [12], pterygium surgery [13, 14], conjunctival surface reconstruction [15], bullous keratopathy [16], chemical or thermal burns [17], ocular surface reconstruction with or without limbal stem cell grafting [18], and in patients with ocular cicatricial pemphigoid or Stevens-Johnson syndrome [19]. However, some complications have been reported with amniotic membrane transplantation such as sub membrane hematoma[20] in the immediate post-operative period, premature degradation of the amniotic membrane, cheese wiring of the amniotic membrane, post- amniotic membrane transplantation microbial infection [21, 22], sterile hypopyon [23] after repeated amniotic membrane transplantation and calcification [24].

Amniotic membrane is available in 5 forms-
    1. Freshly prepared amniotic membrane
    2. Cryopreserved amniotic membrane
    3. Freeze dried amniotic membrane
    4. Prokera
    5. Amniotic membrane extract (AMX)

Freshly prepared amniotic membrane is cheap, easily available and can be prepared any time in the labour room but it is not 100% free from HIV transmission. Cryopreserved and freeze-dried versions are commercially available in the market. They are costly but safe in view of HIV transmission. They are devoid of epithelium which helps in easy migration of host epithelial cells over the basement membrane of amniotic membrane. Prokera is a conformer type device designed by Dr. Tseng. It is made of amniotic membrane with a rigid frame so that it can be inserted in to the conjunctival sac. It covers both the bulbar and palpebral conjunctiva and keeps them apart. It acts as a biologic bandage and very helpful in patients of Stevens Johnson syndrome struggling in the intensive care unit. The device is expensive and has to be changed very frequently.

MATERIAL AND METHODS
Patient Selection
This prospective cross- sectional study comprised of 30 patients having bacterial and fungal corneal ulcers and was conducted at the Regional Institute of Ophthalmology, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, India over a period of one year.

Inclusion Criteria: Patients of any age group presenting with corneal ulcer of
- size more than 5 mm
- deep ulcers i.e. > 50% stromal thickness
- located within 3mm of the visual axis
- Patients with descemetoceles and corneal perforations will also be included

Exclusion Criteria
- Corneal ulcers of size < 5mm depth < 50%
- Associated glaucoma
- Associated chronic dacryocystitis and
- Ulcers with entropion

Detailed history was taken from the patients who fulfilled the inclusion criteria. Detailed slit lamp examination was done in which size of the ulcer, size of infiltration, depth of the ulcer, descemetocele formation and perforation were noted. Digital tonometry and syringing were done. Patients were investigated for blood sugar (fasting and post prandial), complete haemogram and ESR. Corneal scrapings were taken from the base and edge of the ulcers. Gram stain and KOH wet mount were done. Culture was done on blood agar, chocolate agar, thioglycolate broth and sabouraud's dextrose agar. Then treatment was started with broad spectrum topical antibiotics (fortified ceftazidime 50mg/ml and fortified vancomycin 50mg/ml) and topical natamycin 5% according to the clinical appearance. Specific antibiotics were started after reports of culture and sensitivity. Oral antibiotics and antifungals were started in cases of hypopyon, descemetoceles, impending perforations and perforations. Treatment was given for one week. Then amniotic membrane transplantation was performed.

Amniotic Membrane Procurement and processing

The human amniotic membrane was prepared using the standard protocol proposed by Kim [34]. The placenta was obtained from the department of Obstetrics and Gynecology of Pt. B. D. Sharma Post Graduate Institute of Medical Sciences shortly after elective caesarean deliveries who had been screened for HIV, hepatitis B and syphilis in the first trimester in antenatal clinic. Placentas from vaginal deliveries or subsequent to premature rupture of membranes were known to be contaminated and were not considered. The maternal donor was again screened serologically for human immunodeficiency virus (HIV) at the time of caesarean section. This placenta was cleaned with balanced salt solution containing 50 µg/ml ceftriaxone, 50 µg/ml streptomycin, 100 µg/ml amikacin and 2.5 µg/ml amphotericin B (Ceftriaxone and amikacin were used in places of penicillin and neomycin respectively which were used earlier by Kim et al because these drugs are not available now a days. Also, the mechanisms of action are same and the spectrums of antibacterial activity are comparable). The amniotic membrane was separated from the chorion by blunt dissection through the potential spaces between the two tissues. This tissue was transported to the eye OT in a sterile tray and used on the same day.

Surgical Technique

Amniotic membrane transplantation was performed in eye OT under aseptic conditions. Informed consent was obtained from all the patients prior to surgery. Uncooperative patients were given peribulbar anesthesia and topical anesthesia (0.5% proparacaine) was used for young cooperative patients. The necrotic tissues at the base of the ulcer were debrided and sent for culture and sensitivity. The rolled-up edge of the ulcer or the loosely adhered epithelium adjacent to the ulcer was also removed. The amniotic membrane was trimmed to fit the shape of the ulcer and placed with its epithelial side up. Then the amniotic membrane was secured with 10-0 nylon suture (5 cases with interrupted and 25 cases with continuous suture) with the suture knots buried. A double layer amniotic membrane transplantation was done for 2 ulcers of depth more than $3/4^{th}$ of cornea with descemetocele. For a double-layered AMT, one layer of amniotic membrane was overlaid on another layer, both with the epithelial side up. After trimming to fit the shape of the ulcer, both layers were secured in place with 10-0 nylon continuous suture. At the end of the surgery Gatifloxacin ointment was applied and eye was patched for 6 hours, then administration of antimicrobial agents was resumed. Patients were examined and the parameters described below were recorded on 1st post-operative day, first week, second week, fourth week and eighth week.

The following parameters were recorded before and after AMT-
1. Pain
2. Congestion
3. Best corrected visual acuity
4. Size of the ulcer
5. Anterior chamber depth
6. Anterior chamber reaction

Amniotic membrane status and any repetition of AMT were noted.

The qualitative parameters (For calculation purposes) were graded as below.

Pain (Verbal Descriptor Scale [35, 36])	
No pain	1
Some pain	2
Considerable pain	3
Severe pain	4
Unbearable pain	5
Congestion	
No congestion	0
Mild (up to 2mm from limbus)	1
Moderate (from 2mm to mid bulbar conjunctiva)	2
Severe (beyond mild bulbar conjunctiva)	3
Visual acuity	
PL (– ve) or PR inaccurate	0
PL (+ve), PR accurate	1
HM+	2
CFCF	3
(>CFCF– 3/60)	4
(>3/60 – 6/60)	5
(>6/60 – 6/18)	6
> 6/18	7

AC reaction	
No reaction to 0.5+ (1-5 cells per 1mm x 1mm slit beam)/ not visible	0
1+ (6-15 cells per 1mm x 1mm slit beam)	1
2+ (16-25 cells per 1mm x 1mm slit beam)	2
3+ (26-50 cells per 1mm x 1mm slit beam)	3
4+ (>50 cells per 1mm x 1mm slit beam)	4
Hypopyon up to 1 mm	5
Hypopyon >1-2 mm	6
Hypopyon > 2mm	7
AC depth	
Normal	1
Shallow	2
Flat	3

The results were compiled, tabulated and compared statistically. The pre and post- operative values of the parameters were analyzed by using student's paired t- test. The results were considered statistically significant at P-value < 0.05, highly significant at P-value < 0.01 and very highly significant at P-value < 0.001.

OBSERVATIONS AND RESULT

AGE DISTRIBUTION: Maximum age was 96 years and minimum was 17 years. Most of the patients were between 51 –70 years with mean age of 60.4±14.40 years.

SEX DISTRIBUTION: No significant different in gender distribution among patients of corneal ulcers. 54% of total were males and 46% were females.

DISTRIBUTION OF TYPE OF ULCER: 54% of the ulcers were bacterial whereas fungal and mixed corneal ulcers consisted of 23% each.

DISTRIBUTION OF TYPES OF AMT: 47% of AMT was done by overlay technique and 53% was by inlay technique.

Table-1: Mean and Standard Deviation of Preoperative Parameters

|	PAIN	CONGESTION	VA	ACR	ACD	ULCER SIZE
MEAN ± SD	3.70± 0.585	2.83 ± 0.372	2.166 ± 0.778	3.5 ± 2.578	1.233 ± 0.667	32.610 ± 11.179

(VA= visual acuity, ACR = anterior chamber reaction, ACD = anterior chamber depth)

Table-2: Comparison of Pre and Post- Operative Pain

	PAIN PRE-OP	PAIN 1ST POD	PAIN 1ST WK	PAIN 2ND WK	PAIN 4TH WK	PAIN 8TH WK
MEAN ± SD	3.70 ± 0.585	2.033 ± 0.764	1.466 ± 0.819	1.166 ± 0.461	1.066 ± 0.253	1.033 ± 0.182
'P' VALUE		<0.001	<0.001	<0.001	<0.001	<0.001

Table-2 shows that most of the patients presented with severe pain which was reduced drastically in the immediate post-operative period. It was statistically significant from the first post-operative day with P <0.001.

Graph-1 shows the significant decrease in pain from the 1st post-operative day and gradually decreasing thereafter.
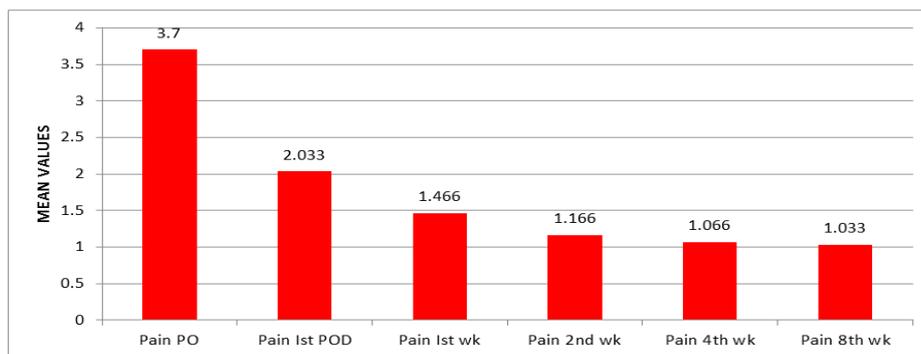
Graph-1: showing gradual reduction in pain postoperatively as compared to preoperative values
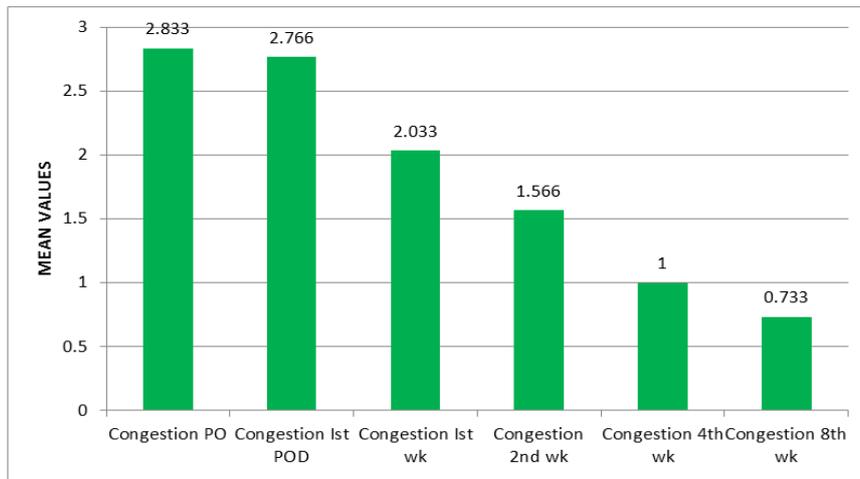
Table-3: Comparison of Pre and Post-Operative Congestion

	CONGESTION PRE-OP	CONGESTION 1ST POD	CONGESTION 1ST WK	CONGESTION 2ND WK	CONGESTION 4TH WK	CONGESTION 8TH WK
MEAN ± SD	2.833 ± 0.372	2.766 ± 0.430	2.033 ± 0.718	1.566 ± 0.678	1.00 ± 0.643	0.733 ± 0.583
'P' VALUE		0.080	<0.001	<0.001	<0.001	<0.001

Table-3 depicts comparative results of levels of congestion in pre and post-operative follow up periods. Average congestion was near severe before AMT. The difference between the congestion level of pre-operative period and 1st post-operative day was found insignificant statistically (P=0.080). This reduced significantly from the very first post-operative week with P <0.001.

Graph-2 indicates the gradual decrease in congestion after amniotic membrane transplantation.

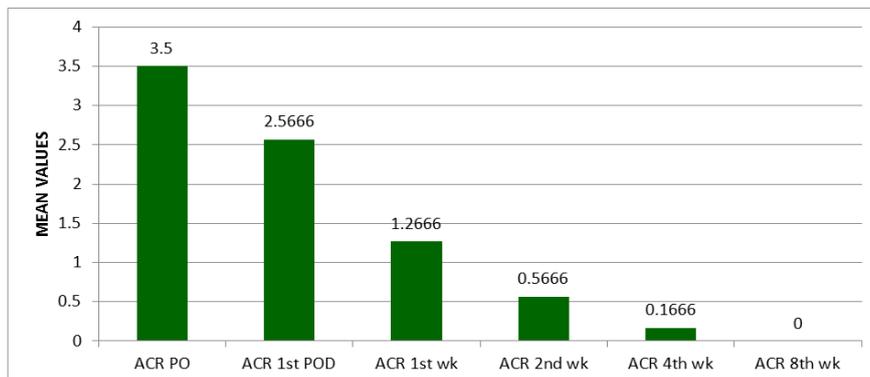**Graph-2: Showing gradual reduction in congestion postoperatively as compared to preoperative values**

Table-4: Comparison of Pre and Post-Operative Anterior Chamber Reaction

	ACR PRE-OP	ACR 1ST POD	ACR 1ST WK	ACR 2ND WK	ACR 4TH WK	ACR 8TH WK
MEAN ± SD	3.50 ± 2.578	2.566 ± 2.824	1.266 ± 2.083	0.566 ± 1.524	0.166 ± 0.912	0.000 ± 0.000
'P' VALUE		0.003	<0.001	<0.001	<0.001	<0.001

Table-4 shows comparison between pre and post-operative anterior chamber reaction (ACR). It was significantly reduced with P=0.003 in the first post-operative day and decreased further in the next follow up periods with P <0.001.

Graph 3 depicts the gradual decrease in anterior chamber reaction after AMT.

Graph-3: Showing gradual reduction in anterior chamber reaction (ACR) postoperatively as compared to preoperative values
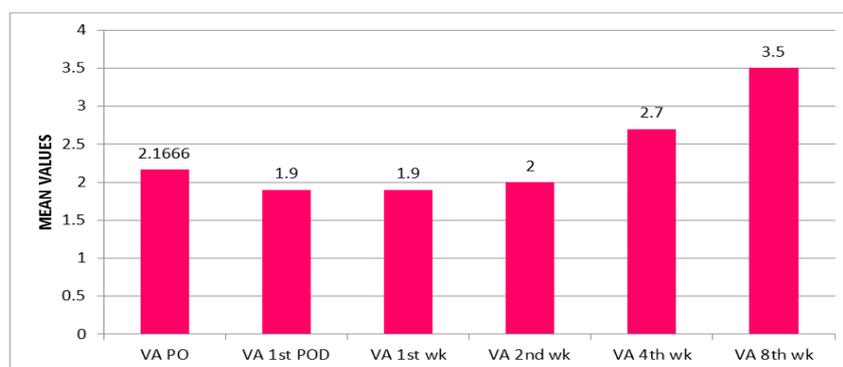
Table-5: Comparison of Pre and Post-Operative Visual Acuity

	VISUAL ACUITY PRE-OP	VISUAL ACUITY 1ST POD	VISUAL ACUITY 1ST WK	VISUAL ACUITY 2ND WK	VISUAL ACUITY 4TH WK	VISUAL ACUITY 8TH WK
MEAN ± SD	2.166 ± 0.778	1.90 ± 0.402	1.90 ± 0.402	2.00 ± 0.587	2.70 ± 1.022	3.50 ± 1.279
'P' VALUE		0.028	0.028	0.129	0.001	<0.001

Table-5 shows the comparison between pre and post- operative visual acuity. The average preoperative visual acuity was hand movement (HM). There was significant decrease in visual acuity till I[st] week follow (P=0.028). At 2nd week the decrease in visual acuity was not significant (P=0.129) i.e. vision was increased as compared to Ist week follow up. From 4th week follow up, vision started increasing significantly to an average of more than counting finger close to face to 3/60 in 8 weeks (P=0.001 at 4th week and <0.001 at 8th week).

Graph-4 shows the decrease in visual acuity till 2nd week follow up which gradually started increasing from 4th week follow up.


Graph-4: Showing decrease in visual acuity till 2nd week follow up which gradually started increasing from 4th week follow up

Table-6: Comparison of Pre and Post-Operative Ulcer Size

	ULCER SIZE PRE-OP (In mm^2)	ULCER SIZE 1ST POD (In mm^2)	ULCER SIZE 1ST WK (In mm^2)	ULCER SIZE 2ND WK (In mm^2)	ULCER SIZE 4TH WK (In mm^2)	ULCER SIZE 8TH WK (In mm^2)
MEAN ± SD	32.610 ± 11.179	32.610 ± 11.179	28.394 ± 13.018	14.770 ± 15.249	1.283 ± 3.352	0.033 ± 0.182
'P' VALUE		Can't measure	<0.001	<0.001	<0.001	<0.001

Table-6 shows that the mean preoperative ulcer size was 32.61±11.179mm^2 which was gradually reduced to 1.283mm^2 (P <0.001) at 4th week follow up and 0.033mm^2 at 8th week follow up (P <0.001).

Graph-5 indicates gradual decrease in ulcer size from 1st week follow up.
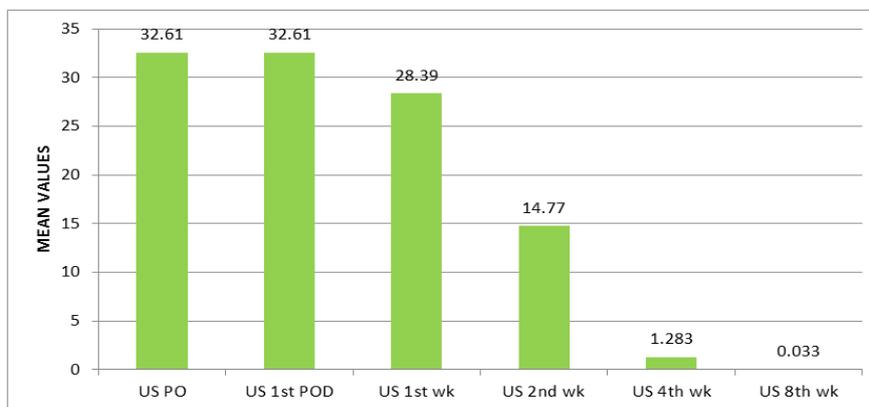
Graph-5: Showing gradual reduction in ulcer size postoperatively as compared to preoperative values

Table-7: Status of Overlay AMT

TOTAL NO OF AMT	DISLODGED	CHEESE WIRING	REMOVED
16	10(62%)	5(31%)	1(7%) at 8th wk
	7 at 2nd wk (43.5%)	1 at 1st wk (6%)	
	3 at 4th wk (18.5%)	4 at 2nd wk (25%)	

Table-7 indicates that 62% of overlay AMT was dislodged (43.5% in 2nd week and 18.5% in 4th week). Cheese wiring was seen in 31% of cases (6% in 1st week and 25% in 2nd week). 7% of AMT was removed at 8th week after healing of the ulcer.

Table-8: Status of Inlay AMT

| TOTAL NO OF AMT	ABSORBED	DISLODGED	RESIDUAL SUB-EPITHELIAL MEMBRANE
18	10(55.5%)	4(22.25%)	4(22.25%)
		2 at 1st wk	
		2 at 4th wk	

Table-8 shows the status of inlay AMT. 55.5% absorbed, 22.25% dislodged and 22.25% remained as residual subepithelial membrane.

Table-9: Healing Period of Corneal Ulcer

MINIMUM HEALING PERIOD(IN WEEKS)	MAXIMUM HEALING PERIOD(IN WEEKS)	MEAN HEALING PERIOD(IN WEEKS)	STANDARD DEVIATION(IN WEEKS)
2	8	4.6	1.83

Table-9 depicts the mean healing period of the corneal ulcers is 4.6 ± 1.83 weeks.

Procurement of Amniotic Membrane

PATIENT 1
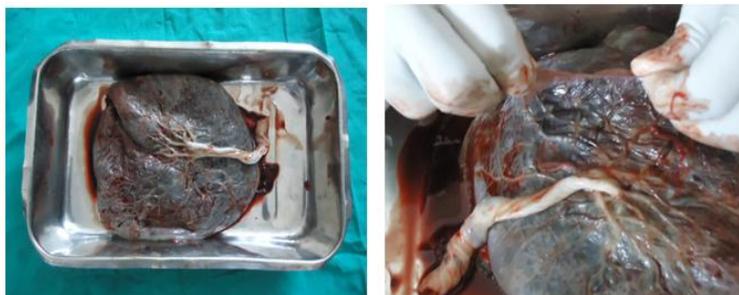
PATIENT 2

preop · post op 1 wk · pre op · post op 1 wk · Post-op 4 wk · Post-op 8 wks · post op 4 wks

PATIENT 3

Pre op

Pre op

Post op 1 wk

Post op 4 wks

PATIENT 4

Pre op

1st post-op day

4th week post-op
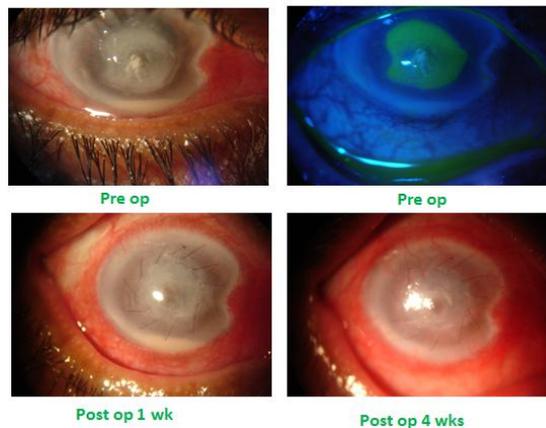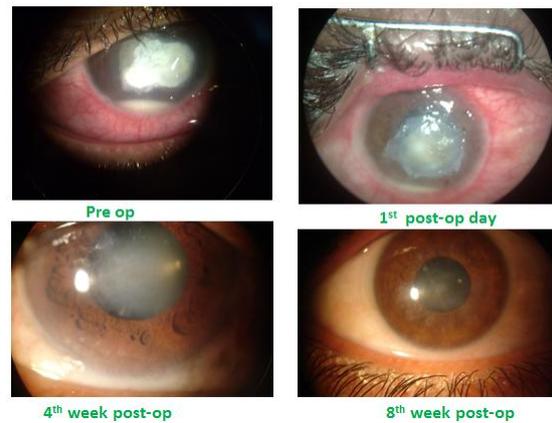
8th week post-op

DISCUSSION

Corneal opacity secondary to corneal ulceration is a major cause of blindness in the developing countries like India. The ultimate aim of treating a corneal ulcer is quick healing with least scarring and vascularization. Most of our patients were between 51 –70 years with mean age of 60.4±14.40 years. Maximum age was 96 years and minimum was 17 years. Among 30 patients 16(54%) were males and 14(46%) were females. There is no significant different in gender distribution. These data are comparable with the study by Hick et al., [25] where thirty-two patients were involved, 16(50%) men and 16(50%) women and the average age of the population was 62 ± 17.8 years (range, 16-85). In our study 16(54%) ulcers were bacterial, 7(23%) were fungal and 7(23%) were mixed corneal ulcers.

Therapeutic Efficacy of Amniotic Membrane Transplantation

1. The most prominent feature of AMT was significant post-operative pain reduction. The mean preoperative pain was 3.70±0.585 which was reduced to 2.033±0.764 in the very 1st post-operative day with P<0.001 and gradually decreased to 1.033±0.182 at 8th week follow up with P<0.001. Sheha et al., [32] performed early sutureless amniotic membrane transplantation for severe bacterial keratitis in which pain was significantly reduced from the first post- operative day. Gicquel et al., noted a significant decrease in the pain score from the admission day to shortly after AMT (at day 3: median, 2; range, 1-3) in severe bacterial keratitis [37]. So the current study is comparable with the previous studies in pain reduction after AMT.

2. The congestion was decreased significantly from 1st week follow up with P <0.001 and the anterior chamber reaction was also decreased significantly from the 1st post- operative day with P=0.003. These results are comparable with the previous studies [29, 32] where inflammation was markedly reduced in all cases.

3. Anterior chamber depth was not changed significantly (P=0.080) after AMT. 24 patients had normal anterior chamber depth preoperatively. In one patient (case 3) anterior chamber was not visible pre and post-operatively. In case 21 anterior chamber depth did not improve and remained flat post-operatively because of anterior synechia. Out of 4 descemetoceles 3 patients (case 12, 16, 21) did not show any increase in anterior chamber depth because of anterior synechia. In case 14 shallow chamber became normal after AMT. In contrast Kim et al., [26] achieved well formed deep anterior chamber in all patients after fibrin glue (FG)-assisted augmented amniotic membrane transplantation in corneal perforations of more than 2 mm in diameter in a series of 10 cases. The reformation of anterior chamber in our study was not possible because of anterior synechia.

4. The average preoperative visual acuity was 2.166±0.778 i.e. hand movement. There was significant decrease in visual acuity till 1st week follow up (P=0.028). At 2nd week the decrease in visual acuity was not significant (P=0.129) i.e. vision was increased as compared to 1st week follow up. From 4th week follow up vision started increasing significantly to an average of more than counting finger close to face to 3/60 in 8 weeks (P=0.001 at 4th week and <0.001 at 8th week). Immediate decrease in vision was probably due to the membrane itself because the fresh amniotic membrane is not as transparent as the cryopreserved one. As the membrane started melting the visual axis became clear thereby increasing the visual acuity gradually from 4th week follow up. 2 patients (6.66%) had shown final visual acuity less than preoperative value probably because of double layer AMT (cases 14, 24). 4 patients (13.32%) retained the same visual acuity as preoperative value (cases 14,21,24,26). 8

patients (26.66%) preserved useful vision of $\geq$ 3/60 without subsequent surgeries (cases 1, 2, 8, 10, 15, 19, 25, 30). The best final visual acuity was 6/24 (cases 2 and 30). Chen et al[31] performed AMT in 23 patients for persistent corneal ulcers and perforations in acute fungal keratitis and reported that the final visual acuity improved in 17 cases (73.91%), worsened in 2 cases (8.69%), and remained unchanged in 4 cases (17.38%). Twelve of the 23 eyes (52.2%) in this study preserved useful vision (20/400 and better) with or without subsequent surgeries. Kim et al., [38] reported improvement of vision in 16 of 21 (76.19%) patients after amniotic membrane transplantation in infectious corneal ulcers and rest needed further management for improvement of vision. So visual outcome in our study is less than the previous studies but further management like optical penetrating keratoplasty and longer period of follow up may improve the visual acuity.

5. The mean preoperative ulcer size was $32.610\pm11.37mm^2$ which gradually decreased to 28.394 ± 13.018 mm^2, 14.770 ± 15.249 mm^2, 1.283 ± 3.352 mm^2 and 0.033 ± 0.182 mm^2 in 1st, 2nd, 4th and 8th week respectively with a P value <0.001 at 1st week onwards. This shows very significant reduction in ulcer size after AMT. All the corneal ulcers were completely healed at the end of 8th week follow up except one (case 22) in which ulcer of 1x1mm remained unhealed and epithelialized completely in next 1 week without repetition of AMT. 3 (10%) ulcers were completely healed at 2nd week follow up, 21 (70%) at 4th week follow up and 5(16.66%) at 8th week follow up. The average healing period of corneal ulcer was 4.6 ± 1.83 weeks. Compared to these results Gicquel et al achieved epithelial healing between 8 and 45 days (mean, 25.5 ± 9.7 days) after AMT in severe bacterial keratitis [35]. Hick et al., reported that 90% of ulcers epithelialized within 3.6 ± 1.6 weeks after performing AMT in corneal ulcers and perforations [25]. Many other previous studies [39-42] also show comparable results with the present study in view of epithelial healing period. 2 cases of descemetocele were healed with normal contour of the cornea at the end of 8th week. No ulcer or descemetocele needed therapeutic or tectonic penetrating keratoplasty. No recurrence of infection was noted. These results are comparable with Kruse et al., [29] who achieved successful results of multilayer AMT in descemetocele.

6. In literature, approximately 25-35% of cases of fungal keratitis require surgical interventions (lamellar or penetrating keratoplasty) at the acute stage to prevent perforation or spreading of infection [43-47]. But in our study no case of treatment failure had come across requiring therapeutic or tectonic keratoplasty.

Complications

The most common complication observed was dislodgement of the membrane requiring repetition of AMT. Out of 16 overlay AMT 5(31%) showed cheese wiring (1 at 1st week and 4 at 2nd week follow up which were subsequently dislodged in the next visit), 10 (62%) were dislodged (7 in 2nd week and 3 in 4th week follow up) and. 3 cases required repeat AMT. Out of 18 inlay AMT, 4 (22.25%) dislodged (2 at 1st week and 2 at 4th week follow up) and 4 (22.25%) patients suffered from residual subepithelial membrane in the visual axis reducing the final visual acuity. In comparison Shi et al., [28] reported that superficial amniotic membrane patches dissolved or shed on postoperative day 7–10 in multilayer AMT for the treatment of necrotizing herpes simplex stromal keratitis. Kim et al., [48] reported early detachment of the membrane in 4% of cases. One patient (case 11) presented with two bullae at the inferior margin of the membrane at 2nd week follow up which were punctured with 26 G needle. They reappeared in the next visit and again punctured. At the final visit there were no bullae. This complication has not been described in any other studies. Unlike other studies [20-24] no other complications like sub membrane hematoma, microbial infection, sterile hypopyon or calcification was noted.

CONCLUSION

Amniotic membrane transplantation can be used as an adjuvant treatment in bacterial and fungal corneal ulcers for early healing, less vascularisation and scarring and better final visual outcome.

Patient comfort is greatly increased because pain is significantly decreased from the immediate post-operative period. It allows postponement of corneal grafting until the eye is less inflamed, thereby improving graft survival or even avoiding it altogether, if the result is sufficiently functional. It can avoid therapeutic and tectonic penetrating keratoplasty in advanced corneal ulcers, descemetoceles and perforations. Overlay technique has more chance of graft dislodgement but heals the ulcer with less vascularisation and scarring than inlay technique. Also, there is no chance of residual subepithelial membrane which hampers the final visual acuity. Continuous suture is better than interrupted suture for graft stability. As the freshly prepared amniotic membrane can be easily prepared in the routine operation theatre with minimum expenditure, it is cost-effective and reasonably safe to be used in developing countries like India.

Limitations of the present study
1. Amniotic membrane used in this study was freshly prepared and HIV ELISA of the donor was not done 6 months after delivery. So, there was a remote chance of transmission to the recipient.

2. Culture of the tissue from ulcer margin was not done to rule out the persistence of infection. It was the clinical appearance to decide about healing.

3. It was not always possible to obtain the membrane immediately. So, the patient had to wait till the membrane is not collected.

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