



## Amniotic membrane a boon for resistant corneal ulcer

Shailly Raj<sup>1</sup>, Brijesh Singh<sup>2</sup>, Rajani Rawat<sup>3</sup>, Sonali Singh<sup>4</sup>, Ifsa Sami<sup>5</sup>

Department of Ophthalmology, UPRIMS & RESEARCH, Saifai E,tawah 1-Senior Resident, 2-lecturer, 4-Post Graduate student, 5-PostGraduate student. Department of Obs & Gynae.3-Assistant Professor

**Corresponding Address:** Dr. Shailly Raj, Senior Resident, Department of Ophthalmology, UPRIMS & Research, Saifai E,tawah. Email: [shailly11@gmail.com](mailto:shailly11@gmail.com)

### Abstract:

This is a prospective interventional case series performed in the patients attending in opd of ophthalmology, UPRIMS & R SAIFAI over the period of 1 year. The detailed history, general and ophthalmic examination done on 24 selected resistant corneal ulcer patients and then The amniotic membrane transplantation done in these patients. The subsequent follow up done at the intervals of 1 week,1 month, 3 months and 6 months. Results showed that the amniotic membrane uptake was very good and overall failure rate was very low. All the patients included in the study were stain positive before procedure and 83% of them converts to stain negative after 1 month of transplantation. The vision not improved in early postop period but improved slightly higher than preop value on late follow-ups. Recurrence of keratitis was not present frequently. The beneficial effect of amniotic membrane was due to growth factors, laminin and fibronectin. Amniotic membrane also had mechanical factor to protect regenerating epithelium. It promotes faster healing (epithelization) of ulcer in comparison to other treatment modalities. Hence amniotic membrane transplantation proved a boon for resistant corneal ulcer.

**Key words:** Amniotic membrane Transplantation, Resistant Corneal Ulcer, Descemetocelles, Perforated Corneal Ulcer

### Introduction:

The defect in the integrity of cornea with inflammation is called corneal ulceration. Corneal ulceration is one of the leading causes of blindness in the world[1].In India about 25000 cases of corneal ulcer are reported every year [2] and about 6.8 million persons are having corneal blindness of which most common cause is corneal ulcer[3].

The patients of corneal ulcer present with decreased vision, pain, photophobia, lacrimation and redness. The cardinal corneal sign is a localized or diffuse infiltration of epithelium and stroma. There is some degree of lid edema. Conjunctiva is chemosed and injected. There may be conjunctival discharge. Anterior chamber inflammatory signs are also present with cells, flares and hypopyon in severe cases.

The **diagnosis** of corneal ulcer is based primarily on clinical history and physical examination. Identification of infectious organism should be achieved by examination of stained smears of corneal scraping and culture.

Corneal ulcers are **treated** with topical application of antibiotic eye drops. Higher corneal levels of drug can be established with more frequent application of drops.

The corneal ulcer that does not show any response in two weeks despite proper medical management is called **resistant corneal ulcer**. In these patients use of amniotic membrane transplantation may be a better alternative as it has some biological healing properties in addition with mechanical properties.

**Human amniotic membrane** is derived from the fetal membranes which consist of the inner amnion made up of a single layer of amnion cells fixed to collagen rich mesenchyme which is 6-8 cells thick, loosely attached to chorion [4].

The **factors** by which amniotic membrane delivers its beneficial effects are:-

#### 1. Growth factors [4]

- Epithelial growth factor (E G F)
- Transforming growth factor (T G F) alpha

- Transforming growth factor (T G F) beta<sub>1</sub>, beta<sub>2</sub> and beta<sub>3</sub>
- Human chorionic growth factor (H G F)
- b Fibroblast growth factor (b F G F) etc.

Growth factors help in prolongation of the life span and clonogenicity of epithelial progenitor cells.

**2. Cytokines and protease inhibitors** as interleukin-4, interleukin-6 and macroglobulin-2 are also present. These factors are also responsible for inhibition of inflammatory cells with antiprotease activity[5]

**3. Amniotic membrane also contains laminin and fibronectin** that is similar to those of epithelial basement membrane of cornea[6]. Fibronectin helps in cell migration and cell to substrate adhesion. Laminin helps in adhesion of epithelial cells to stroma.

**4. Amniotic membrane also provides its mechanical factors** by protecting the regenerating epithelium from movement of eyelids.

Thus amniotic membrane transplantation shows following clinical effects:-

- Facilitates epithelialization by acting as a scaffold.
- Maintains normal epithelial phenotype.
- Reduces inflammation.
- Reduces scarring.
- Reduces vascularization.
- Promotes adhesion of epithelium.

Human amniotic membrane is believed to be **non-immunogenic**. It does not express HLA-A, B, C, DR or beta-2 microglobulin [7,8]. Antibodies and cell mediated immune response have not been demonstrated, suggesting its low antigenicity. Therefore the use of systemic immunosuppressive in amniotic membrane graft is not required. In contrast chorion provokes neovascularization and typical rejection phenomenon. So it should be separated.

### Aims and Objectives

1. To study the role of amniotic membrane transplantation in-  
-Non healing corneal ulcers.  
-Descemetocoeles.  
-Microleakages and other leakages <1.5mm in size.
2. To study the failure rate following amniotic membrane transplantation.
3. To study the short term and long term adverse effects of amniotic membrane transplantation in the form of recurrence of keratitis, transparency and neovascularization.

### Materials and Methods

It is a prospective interventional case series which is performed in the patients of resistant corneal ulcer attending the O. P. D. of Department Of Ophthalmology, U.P.RIMS&R,SAIFAI. 24 patients were selected between the periods from October 20013 to October 2014, for amniotic membrane transplantation according to criteria given below.

**Eligibility criteria:** - Patients with corneal ulcer should meet the following criteria:-

1. Persistence of signs and symptoms of corneal ulcer despite the conventional management for two weeks duration treated in this hospital or outside.
2. Patients having corneal ulcer with impending
3. Patients having corneal ulcer with small perforation size less than 1.5 mm. (self sealed).

#### Exclusion Criteria:

Patients with corneal perforations secondary to penetrating eye trauma, active infections, lid abnormalities, lagophthalmos, corneal and conjunctival ischemic conditions as in chemical burn, corneal ulcers in aphakic eyes, large corneal perforation, autoimmune disorders.

#### Pre-Operative Evaluation

**A.** Detailed History (Ocular and Systemic),

**B.** General Examination

**C.** Systemic Examination

**D.** Ocular Examination

1. Visual acuity

2. Torch light and slit lamp examination

a. Examination of eyelids and conjunctiva.

b. Examination of cornea

- Fluorescein staining

- Size and depth of ulcer

- Condition of ocular surface.

c. Examination of anterior chamber

3. Corneal sensation

**E.** Investigations: 1. Blood investigations- Hemoglobin, TLC /DLC, Blood sugar level.

2. Microbiological Examination: - Microscopic examination and culture with sensitivity of sample taken by corneal scraping.

Amniotic membrane was **obtained and processed** with informed consent from all the donors, who were seronegative for hepatitis B, hepatitis C, syphilis and HIV 1 and 2, and underwent elective caesarian section. The placenta was obtained under strictly sterile condition.

Under Topical anesthesia necrotic material and debris from ulcer bed were removed then a suitable piece of amnion was cut and placed on corneal surface with stromal surface in contact with the cornea. In this study 'overlay' technique was

used [9]. The graft was secured in place using 6-0/8-0 vicryl interrupted sutures to the limbus. Post-operatively preservative free antibiotic (Moxifloxacin) eye drop 6 times a day and lubricant eye drop 6 times a day were given.

Follow-ups were done at the period of 1 week, 2 weeks, 1 month, 3 months and 6 months post-operatively. Sutures were removed at 2-4 weeks follow up.

### Post-Operating Evaluation

Ocular Examination

Visual acuity test.

Torch light and slit lamp examination of cornea-

- Re-epithelization of surface
  - Grant /tissue edema
  - Graft /tissue vascularity
  - Melting and retraction of graft
  - Infection and surface necrosis
- c. Suture debris and exposed knots

### Results

**Table 1: Age and sex distribution: -**

Age (Years)	Number of cases (%)	Male (%)	Female (%)
20-30	2 (8.33)	1 (4.17)	1 (4.17)
30-40	3 (12.5)	2 (8.33)	1 (4.17)
40-50	5 (20.83)	3 (12.5)	2 (8.33)
50-60	10 (41.67)	6 (25)	4 (16.67)
>60	4 (16.67)	2 (8.33)	2 (8.33)
Total	24 (100)	14 (58.33)	10 (41.67)

In 24 patients, amniotic membrane transplantation was done. In which 14 patients (58.33%) were male and 10 patients (41.67%) were female. Maximum number of patients (41.67%) was between the age group 50-60 years.

Mean of age- 49.58 years.

Standard deviation of age- 11.6 years.

**Table 2: Indications: -**

Indication	Number of cases (%)
Non healing corneal ulcer	20 (83.33)
Descemetocoeles	3 (12.5)
Perforated corneal ulcer	1 (4.17)
Total	24 (100)

In 24 selected cases, 20 cases (83.33%) were of non healing corneal ulcer, 3 cases (12.5%) were of descemetocoeles and 1 case (4.17%) was of perforated corneal ulcer.

**Table 3: Best corrected Visual Acuity (BCVA): -**

Grade s (BCV A)	Pre-op BCV A (%)	Immed iate post-op BCVA (%)	Aft er 1 we ek (%)	Afte r 1 mont h (%)	After 3 mont hs (%)	After 6 mont hs (%)
PL +nt to HM	20 (83.33)	24 (100)	24 (100)	23 (95.83)	20 (83.33)	18 (75)
HM to 6/60	4 (16.67)	0	0	1 (4.17)	4 (16.67)	6(25)

In selected 24 patients, pre-operative visual acuity of 20 patients (83.33%) was between PL +nt to hand movement and of 4 patients (16.67%) was between hand movement to 6/60. In immediate post-operative period, visual acuity deteriorated (all patients PL to HM) but improved later on.

**Table 4: Epithelialization: -**

Time duration	Stain +ve (%)	Stain -ve (%)
Pre-operative	24 (100)	0
After 1 month	4 (16.67)	20 (83.33)
After 3 months	5 (20.83)	19 (79.17)
After 6 months	6 (25)	18 (75)

After 1 month post-operatively, epithelialization occurred in 20 patients (83.33%), after 3 months, success rate was 79.17% (in 19 patients) and after 6 months, epithelialization was maintained in 18 patients (75%).

**Table 5: Retraction: -**

Time (post-operatively)	Number of cases (%)
1 week	2 (8.33)
2 weeks	2 (8.33)
1 month	23 (95.83)
3 months	24 (100)
6 months	24 (100)

In 2 patients, amniotic membrane was retracted during first week. In maximum patients (23 patients- 93.83%) retraction occurred after 1 month. In 1 patient, amnion was retracted between 1 to 3 months.

**Table 6: Recurrence of keratitis:-**

Time duration	Number of cases (%)
1 month	0
3 months	1 (4.17)
6 months	2 (8.33)

After 1 month post-operative period, recurrence of keratitis was not shown in any patient. After 3 months recurrence occurred in 1 patient (4.17%) and after 6 months it was seen in 2 patients (8.33%).

#### Table 7: Neovascularization: -

Time duration	Number of cases (%)
1 month	3 (12.5)
3 months	8 (33.33)
6 months	15 (62.5)

1 month after amniotic membrane transplantation, neovascularization was seen in 3 patients (12.5%). After 3 months, it was seen in 8 patients (33.33%) and after 6 months in 15 patients (62.5%).

#### Discussion

Treatment of corneal ulcer should be started as early as possible because it causes destruction of normal ocular structures. If not treated properly, it may cause many complications like stromal melting, descemetocoeles formation, perforation of cornea, anterior chamber collapse, adherent leucoma, phthisis bulbi etc.

In this study, we used amniotic membrane transplantation for the treatment of resistant corneal ulcer because it has some biological properties in addition with its mechanical properties. So it should be better alternative than soft contact lens or conjunctival flap surgery.

This study was conducted on 24 patients of resistant corneal ulcer, who had taken conventional medical treatment for more than 2 weeks. Among the 24 patients, 14 patients were male and 10 patients were female. It was similar to epidemiology of corneal ulcer that it is more common in male than female, because male are more exposed to external environment and trauma than female.

The patients were between the age group 20 to more than 60 years. Mean age group of patients was 49.58 years and standard deviation was 11.6 years. The maximum number of patients-10 was between the age group 50-60 years. It was similar to epidemiology of corneal ulcer that it is more common in middle age adults who are more prone to trauma and diabetes mellitus like conditions.

Visual acuity deteriorates much in the patients of corneal ulcer. In selected 24 patients, pre-operative visual acuity of 20 patients (83.33%) was between PL +nt to hand movement and of 4 patients (16.67%) was between hand movement to 6/60. Immediately after the amniotic membrane

transplantation (day 1) visual acuity deteriorated in all patients. After 1 month post-operatively, visual acuity began to improve; and after 4 months, it was improved to pre-operative value. At the period of 6 months post-operatively, the visual acuity was improved [HM to 6/60 in 6 patients (25%)] in successful cases, but not much and required penetrating keratoplasty for improvement of vision results were similar to a study done by **Ferreira De Souza R, Hoffmann-Rummelt C, et al [10]** (2001), in which the visual acuity was  $\leq 20/400$  (6/120) in 60 % eyes preoperatively, but improved to that level in 69 % of patients after 3 months and in 78 % after 6 months of follow-up.

In our study 1 month after the surgery, success rate was 83.33% (ulcer was healed in 20 patients). But at the 3 months and 6 months of follow up, success rate was maintained in 79.17% (19 patients) and 75% (18 patients) respectively. Success rate in this study was nearly similar to study of **Kruse FE, Rohrschneider K and Volcker HE [11]** (1999) studied to evaluate the efficacy of multilayer amniotic membrane transplantation for reconstruction of corneal epithelium and stroma in the context of deep corneal ulcers. They got success in 82% of cases in 1 year follow up. **Hanada K, Shimazaki J, Shimmura S, et al [12]** (2001) studied the use of multilayered amniotic membrane transplantation for severe ulceration of the cornea and sclera. Epithelialization occurred in 72.7% cases in their study in 1 month. In 2001, a study done by **Muraine M, Descargues G, Franck O, et al [13]** showed that their best results of amniotic membrane transplantation were in persistent trophic ulcers of cornea with a success rate of 80% within 4-11 months of follow up.

In this study, overlay technique was used in all patients. In 2 patients, amniotic membrane was retracted during first week. In maximum patients (23 patients-93.83%) retraction occurred after 1 month. In 1 patient, amnion was retracted between 1 to 3 months. It is nearly similar to study done by **Muraine M, Descargues G, Franck O, et al [13]** (2001) in which dissolution of amniotic membrane took place in 2-3 months in most of the cases.

In this study, recurrence of keratitis was not shown in any patient at 1 month post-operative period. After 3 months, recurrence occurred in 1 patient (4.17%) and after 6 months, it was seen in 2 patients (8.33%). **Hanada K, Shimazaki J, Shimmura S, et al [12]** (2001) studied that in some eyes with total corneal limbal dysfunction or autoimmune disorders, recurrence may occur.

In this study, 1 month after amniotic membrane transplantation, neovascularization was seen in 3 patients (12.5%). After 3 months, it was seen in 8 patients (33.33%) and after 6 months in 15 patients (62.5%). This study was akin to the study done by **Ferreira De Souza R, Hoffmann-Rummelt C, et al** [10] in 2001. They found that in those cases, neovascularization was present only in 60% of eyes at 6 month follow up, as compared to more than 90% of cases not on treatment.

#### CONCLUSION

From the observation made in foregoing study "Amniotic Membrane: boon for Resistant Corneal Ulcer" following conclusions have emerged out:-

1. In this study, epithelialization occurred in 75% of patients. So this method is a good surgical method for the treatment of resistant corneal ulcer.

2. Visual acuity deteriorated in immediate post-operative period, but improved later on slightly more than the pre-operative value. Vision may be improved further with keratoplasty.

3. In maximum number of patients (93.83%), amniotic membrane was maintained for more than 1 month. So the rate of amniotic membrane graft uptake is very good and failure is very low.

4. After 1 month post-operative period, recurrence of keratitis was not shown in any patient. However after 6 months of follow up, it was seen in 2 patients (8.33%).

5. Within the 6 months of follow up, neovascularization was present in only 62.5% of patients as compared to more than 90% of cases not on treatment. So amniotic membrane transplantation reduces the rate of neovascularization.

From this study, we concluded that amniotic membrane transplantation is a better surgical procedure for resistant corneal ulcer than its conventional treatment methods.

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