

Evaluation of Fresh Human Amniotic Membrane Transplantation for the Treatment of Corneal Perforation and Impending Corneal Perforation

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Purpose: To determine the outcome of amniotic membrane transplantation for the treatment of corneal perforation and impending corneal perforation.

Material and Methods: This Quasi experimental study was carried out in Ophthalmology department, Benazir Bhutto Hospital from 16th April 2007 to 15th April 2008. Total of 30 patients with either corneal perforation or impending corneal perforation were selected. Freshly prepared amniotic membrane was transplanted over these diseased corneas.

Results: Mean age was 58.7 years with minimum 28 years, maximum 75 years and standard deviation of ± 12.04 , post op hospital stay was 1 week in 28 (93.3%) patients and 2 weeks in 2 (6.7%) patients with mean of 1.07 and standard deviation of ± 0.25 , duration for which graft remained intact was minimum 1 week to maximum 10 weeks with mean of 3.97 weeks and standard deviation of ± 2.40 . Patients were evaluated for relief of symptoms, reduction in conjunctival inflammation, epithelial healing and globe preservation. 28 patients (93.3%) showed improvement in symptoms, reduction in conjunctival inflammation, epithelial healing and globe preservation.

Conclusion: Amniotic membrane is a useful material in treating patients with corneal perforation and impending corneal perforation, by relieving the symptoms, healing the lesion, control of infection and ensuring globe preservation.

Amniotic membrane, the innermost layer of placenta, was first used along with the chorion as a biologic membrane to promote healing of the skin burns in 1910¹. In ophthalmology, it was used in 1940, for the management of conjunctival defects¹. Its revival in 1990 was due to its ability to reduce ocular surface inflammation and scarring, promoting rapid epithelialization due to the presence of growth factors and antimicrobial properties¹. In 1995, amniotic membrane transplantation was used for ocular surface reconstruction of severely damaged rabbit corneas² and since that experimental study, amniotic membrane transplantation has been used for

different ocular surface disorders. As far as cornea is concerned, amniotic membrane acts as a biological contact lens, when transplanted over thin or perforated corneas³. It may be considered as an alternative method for treating persistent epithelial defects and sterile ulceration that are refractory to conventional treatment and before considering treatment by conjunctival flaps or tarsorrhaphy⁴.

Amniotic membrane graft can be used as an effective biomaterial to improve wound healing in corneoscleral ulcerations⁵. It has also been found to be effective in promoting epithelialization and preventing corneal perforations in acute fungal keratitis and there

is no risk of rejection as surface cells of amnion do not express HLA A,B,C or beta 2-microglobulin and so make it an excellent grafting material⁶⁻⁸.

MATERIAL AND METHODS

It was a Quasi experimental study, carried out in department of ophthalmology, Benazir Bhutto Hospital during the period 16th April 2007 to 15th April 2008. Non-probability convenience sampling was done and 30 patients with corneal perforation or impending corneal perforation, regardless of age and gender, were included in the study.

On day of amniotic membrane transplantation, human placenta was harvested from elective cesarean section delivery with no history of Rh incompatibility, hepatitis B, C and HIV, from Gynae department Benazir Bhutto Hospital. Under aseptic measures, placenta was washed thoroughly with antibiotic solution containing Inj. Streptomycin 50 µg/ml, Inj. Gentamicin 100 µg/ml, Inj. Benzyl penicillin 50 µg/ml. While still attached to the placenta, amniotic membrane was separated from chorion and continuously irrigated with the antibiotic solution, till the debris was removed completely and membrane became almost transparent. The freshly prepared membrane was then transplanted over the diseased corneas.

While applying membrane over the cornea, it was placed with the epithelial side up i.e. away from lesion and stromal side of the membrane facing the lesion and anchored with 10/0 nylon.

Either inlay (only the lesion is covered), overlay (whole cornea), or filler (multilayered) technique was used according to the extent and severity of lesion, followed by bandage contact lens application over the graft, which was kept for at least two weeks.

Post operatively, patients were to be kept in hospital for one week, so that daily assessment could be done. 28 patients who started showing improvement were kept for 1 week and 2 patients who didn't show response, were kept for 2 weeks and ultimately were given other treatment modalities.

Post operative medication included topical antibacterials, antifungals and steroids. Parameters assessed were reduction in pain, redness, watering, Reduction in conjunctival inflammation and corneal epithelial healing. This assessment was made daily for 1st week, weekly for next 3 weeks and monthly for next 2 months. So the final conclusion used to be made

at the end of 3 months or even earlier if infection control and complete healing had been achieved earlier, that whether the globe preservation has been achieved or not. It was noted that graft either disintegrates, or becomes part of ocular tissue or in some cases, we removed the graft ourselves when epithelial healing was achieved so duration for which it remained intact, was also noted.

Data was converted into variables and entered in SPSS version 10.

Descriptive statistics were used to calculate mean and standard deviation for age, post operative stay in hospital and the duration for which graft remained intact. Frequencies were calculated for age, symptoms, inflammation, epithelial healing, globe preservation and duration for which graft remained intact.

RESULTS

This was a Quasi experimental study carried out, on 30 subjects suffering from either corneal perforation or impending corneal perforation.

Minimum age was 28 years, maximum 75 years with mean 53.87 years and standard deviation of ± 12.04 . Among these 30 patients, 17(56.7%) were males and 13 (43.3%) were females.

In 12 patients (40%) inlay technique, in 17 patients (56.7%), overlay technique and multilayered (2 layers) in 1 patient (3.3%) was used.

Minimum duration for which graft remained intact was 1 week and maximum 10 weeks with the mean of 3.97 week and standard deviation of ± 2.40 . Details are shown in following table:

Minimum hospital stay was 1 week, which was in 28 patients (93.3 %) and maximum was 2 weeks in 2 complicated cases (6.7%) with mean of 1.07 weeks and standard deviation of ± 0.25 .

As far as improvement in symptoms is concerned, following results were obtained:

Globe preservation by either control or prevention of infection):

In 28 patients (93.3%) globe was preserved due to amniotic membrane transplantation. In rest of two patients 1 underwent tarsorrhaphy in 2nd week and later on his keratoplasty was done. In 2nd patient, evisceration was done in 2nd week.

Table 1: Duration for which graft remained intact

Weeks	Frequency of Patients n (%)	Cumulative Percent
1	4 (13.3)	13.3
2	4 (13.3)	26.7
3	7 (23.3)	50.0
4	7 (23.3)	73.3
5	2 (6.7)	80.0
6	1 (3.3)	83.3
7	1 (3.3)	86.7
8	2 (6.7)	93.3
9	1 (3.3)	96.7
10	1 (3.3)	100.0
Total	30 (100.0)	

Table 2: Reduction in pain against time

	No	Mild to Moderate n (%)	Marked n (%)
	2 (6.7%)	—	28 (93.3)
End of 2 nd Week	—	—	28 (93.3)
End of 3 rd Week	—	—	28 (93.3)
End of 4 th Week	—	—	28 (93.3)
End of 2 nd Month	—	—	28 (93.3)
End of 3 rd Month	—	—	28 (93.3)

Table 3: Reduction in watering against time

	No	Mild to Moderate n (%)	Marked n (%)
End of 1 st Week	2 (6.7%)	22 (73.3)	6 (20)
End of 2 nd Week	—	4 (13.3)	24 (80)
End of 3 rd Week	—	2 (6.7)	26 (86)
End of 4 th Week	—	—	28 (93.3)
End of 2 nd Month	—	—	28 (93.3)
End of 3 rd Month	—	—	28 (93.3)

Table 4: Reduction in redness against time

	No	Mild to Moderate n (%)	Marked n (%)
End of 1 st Week	2 (6.7%)	28 (93.3)	—
End of 2 nd Week	—	28 (93.3)	—
End of 3 rd Week	—	3 (10)	25 (83.3)
End of 4 th Week	—	1 (3.3)	27 (90)
End of 2 nd Month	—	—	28 (93.3)
End of 3 rd Month	—	—	28 (93.3)

Table 5: Reduction in conjunctival inflammation against time

	No	Mild to Moderate n (%)	Marked n (%)
In 1 st Week	2 (6.7%)	28 (93.3)	—
End of 2 nd Week	—	28 (93.3)	—
End of 3 rd Week	—	4 (13.3)	24 (80)
End of 4 th Week	—	—	28 (93.3)
End of 2 nd Month	—	—	28 (93.3)
End of 3 rd Month	—	—	28 (93.3)

Table 6: Epithelial healing against time

Weeks	No. of Patients n (%)
End of 1 st Week	3 (10)
End of 2 nd Week	9 (30)
End of 3 rd Week	18 (60)
End of 4 th Week	23 (76.7)
End of 2 nd Month	27 (90)
End of 3 rd Month	28 (93.3)

DISCUSSION

In our daily practice we encounter many cases with hopeless corneal epithelial defects, perforations and impending perforations, which are resistant to most available conventional treatment options. This study

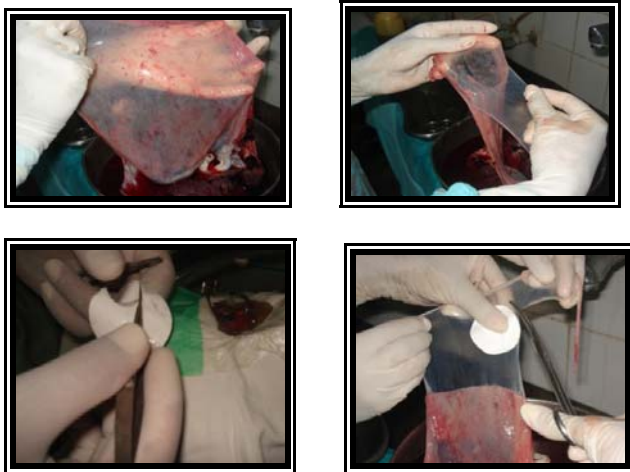
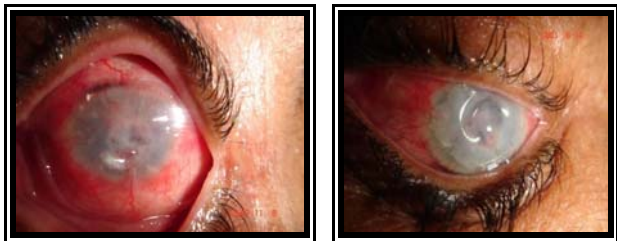
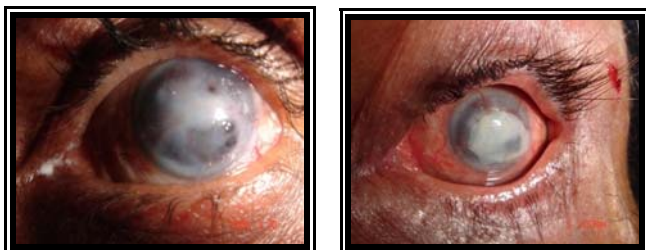


Fig. 1: Preparation of human amniotic membrane



A: After (overlay technique) B: Healed cornea

Fig. 2: Right eye, Central corneal perforation with amniotic membrane graft



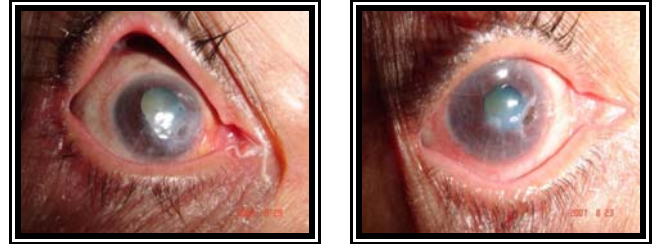
Perforated cornea with intact AM graft B: Healed cornea

Fig. 3: Left eye, Corneal Perforation

was an effort to treat these cases with the use of amniotic membrane grafting in a setting where there was ample supply of fresh amniotic membrane but which lacked proper harvesting and storage facilities in an endeavor to introduce an old technique with relatively newer interests.

The average duration for which the graft remained intact was 4 weeks varying between 1 and 10 weeks.

This was the time, which actually was required for epithelialization to be completed. This duration was almost the same as found out by Lee and Tseng in a similar study⁴.



A: Perforation, cataract B: Incorporating graft



C: Healed cornea with implanted IOL.

Figure 4: Right eye, an eye with eccentric corneal perforation.

The two parameters, redness and conjunctival inflammation were directly related to each other and of course associated with the state of wound healing. At the end of the 2nd month 93.3% had marked improvement in both redness and inflammation. These findings of marked improvement in inflammation associated with healed corneal surface are consistent with what Ma, David Hui-Kang et al⁵.

The anti-inflammatory effect were established by Chen-Hung Chi and associates when eyes with acute fungal keratitis and associated corneal perforations improved after amniotic membrane transplantation⁶.

In this study, the freshly prepared amniotic membrane was used. Most clinical experiences have been with properly preserved tissue at -80 degree Celsius in glycerol solution and flattened onto nitrocellulose filter paper⁹. However, several authors have described the use of freshly prepared human amniotic membrane and found that amniotic membrane transplantation promoted epithelial healing, reduced inflammation, increased comfort, and decreased severity of vascularisation. They did not find any infectious, inflammatory, or toxic

reactions.^{10,11} The cases were generally complication free, as was expected from our prior knowledge from studies on use of freshly prepared amniotic membrane to heal corneal lesions^{10,11}. There have been reports of development of a sterile hypopyon and in some cases, infection¹². No such problem was encountered in this study.

Although fresh tissue may be associated with a higher risk of blood borne diseases¹³, the authors felt that freshly prepared tissue may be important in ocular surface restoration in developing countries.

One of the parameters that was selected to judge wound healing in this study was improvement in symptoms. There was improvement in pain in 93% cases at the end of the first week which was not only a result of a bandage like effect providing a cover to the exposed nerve endings, it actually also signified a good healing response. This benefit of relieving pain when using amniotic membrane in treating corneal diseases has been seen in study of Parasad JK, et al¹⁴. There was also reduction in the amount of watering which again was a collateral advantage of wound healing and the bandage effect. At the end of the 4th week equal number of patients (93.3%) showed a marked reduction in pain and watering.

The prime objective of treating all these eyes was to ensure an optimal epithelial healing of the initial defect and to secure the integrity of the globe. 30% of the eyes achieved epithelial healing by the end of 2nd week, 76.6% at the end of the 4th week and 90% after the 2nd month. 28 out of 30 eyes eventually had complete epithelial healing. One of the patients had a severe corneal melting disorder. In fact, the perforation that he developed exhibited initial recovery with amniotic membrane grafting, his iris got adherent to the posterior corneal surface, developed raised IOP, worsening of the lesion for which a tarsorrhaphy was performed. The perforation got sealed but the cornea later became staphylomatous. A tectonic / cosmetic / therapeutic penetrating keratoplasty was eventually performed. The other case that failed to recover developed infection, continued corneal melting and endophthalmitis, ultimately requiring evisceration. If this was the lowest point in the duration of this study, the highest point came when an only eyed patient was able to see 6/12 after recovering from a corneal perforation through an amniotic membrane transplant and later extraction of a mature complicated cataract and IOL implantation.

What we ultimately achieved in 28 out of the 30 eyes that were selected for the amniotic membrane

transplantation was globe preservation. Taking into account 90% globe preservation by Lee⁶, considering the presenting condition of the eyes, and where Azuara - Blanco failed to show improvement in any one of the 5 eyes with either corneal perforation or impending perforation when they used amniotic membrane transplantation as a treatment modality¹⁵, 93.3% success rate in our study was more than satisfactory.

CONCLUSION

Amniotic membrane transplantation is an important tool for the healing of corneal perforations and impending corneal perforations. Healing of these corneas decreases the distressing symptoms associated with these corneal lesions and helps in globe preservation.

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