NON-PRESERVED HUMAN AMNIOTIC MEMBRANE TRANSPLANT IN OCULAR SURFACE RECONSTRUCTION- A SURGICAL EXPOSURE IN WESTERN ODISHA

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ABSTRACT

BACKGROUND

Human amniotic membrane (AM) is the inner layer of the fetal membranes and consist of the epithelium, basement membrane and stroma. The AM has anti-inflammatory, anti-fibrotic, anti-angiogenic as well as anti-microbial properties. Because of its transparent structure, lack of immunogenicity and the ability to provide an excellent substrate for growth, migration and adhesion of epithelial corneal and conjunctival cells, it can be used for ocular surface reconstruction in many ocular pathologies including corneal disorders associated with limbal stem cell deficiency, surgeries for conjunctival reconstruction, as a carrier for ex vivo expansion of limbal epithelial cells, glaucoma surgeries and scleral melts and perforations. AM transplantation is a very useful armamentarium in the hands of the ophthalmic surgeons for treating a variety of ocular surface disorders caused by various Chemical, acids and alkali and also endogenous causes like severe dry eye, Steven Johnson Syndrome etc affect the ocular surface as it is a very sensitive and dynamic structure.

The aim of the study is to evaluate the efficacy of Non-Preserved Human Amniotic Membrane Transplantation in ocular surface disorders with respect to re-epithelialisation, corneal clarity, degree of neovascularisation and visual outcome and compare its efficacy with that of preserved Amniotic Membrane Transplant.

MATERIALS AND METHODS

40 eyes of 36 patients were subjected to amniotic membrane transplant from July 2016 to January 2018. Non-preserved amniotic membrane was employed to cover corneal ulceration due to dry eye syndrome (12 eyes), non-healing corneal ulcers (12 eyes), corneal epithelial defect due to Steven Johnson Syndrome (8 eyes), conjunctiva and corneal epithelial defect due to alkali burn (8 eyes). Amniotic membrane sutured to surrounding conjunctiva using 10-0 silk with interrupted suture. Patients were followed up to 6 months. Sutures removed on 31st day of transplant.

RESULTS

Out of 40 eyes of 36 patients with mean age group of 32.5 years, non-healing corneal ulcer (8 eyes; 20%), dry eye syndrome (8 eyes; 20%) and Steven Johnson syndrome (4 eyes; 10%) showed resolution of epithelial defect and stabilization of neo vascularisation. 4 eyes (10%) of alkali burn responded favourably but retransplantation of Amniotic membrane to 2nd eye (10%) of the same patient, on 45th day had to be done. Over all 4 eyes of Steven Johnson syndrome, 4 eyes of dry eye syndrome, 4 eyes of persistent sterile corneal ulcer failed due to unrelated bacterial keratitis. Corneal clarity and improved visual acuity were observed in 60% of patients on day 31st. Transplantation in 16 eyes (40%) failed and was sent to higher center for further management.

CONCLUSION

Non-preserved human amniotic membrane transplantation is a safe and equipotent procedure as compared to preserved amniotic membrane in ocular surface reconstruction where facilities for preservation are not available and continuing medical management bears higher risk.

KEYWORDS

Non-preserved Amniotic Membrane, Dry Eye Syndrome, Non-healing Corneal Ulcer, Steven Johnson Syndrome.

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BACKGROUND

Amniotic membrane is the innermost layer of placenta consisting of thick basement membrane and an avascular stromal matrix. The basement membrane of amniotic membrane resembles that of conjunctival basement membrane.

It has a unique property of being an ideal substrate for the growth of epithelial progenitor cells of conjunctiva and cornea by prolonging their life span, maintaining their slow cycling and thus clonigenecity. The stromal matrix of membrane can also exclude inflammatory cells by rendering them into rapid apoptosis. Thus, amniotic membrane transplant can be used for reconstruction of lost ocular surface integrity due to variety of insults to the eye. This article is an effort to analyse the success of non-preserved amniotic membrane transplant in eyes with ocular surface disorder where facilities for preservation of amniotic membrane are not available and medical management of affected eyes showed dwindle response. Amniotic membrane transplantation has been used in many medical conditions not only ocular, like in biological bandage for dressing burns and non-healing skin ulcers, surgical reconstruction of mucous membranes. Ophthalmology used Amnion in 1940 which was 1st reported by Rotth for the treatment of conjunctival tissue loss.[1] In 1946, Sorsby and Symons[2] reported on transplantation of Amnion to treat chemical burns. In 1995, Kim and Tseng[3] published on the use of AMT in ocular surface pathologies based on their work on animals. These authors utilized human amniotic membrane that was preserved in 100% glycerol and stored at 4° C.[3] In 1997, Tseng and his colleagues reported on use of amniotic membrane preserved in 50 % Dulbecco's modified Eagle's medium and 50% glycerol and stored at -80° C in Human subjects.[4] This method of preservation and storage of amniotic membrane has since been used by many others. Recently, freeze dried vacuum packed amniotic membrane that can be stored at room temperature alone is reported to be used.[5] Amniotic membrane can be categorized to have three basic functions.[6] First, it acts as a biological bandage which protects large areas of the underlying ocular surface in conditions like extensive chemical burns. The epithelium also heals underneath the amniotic membrane in these circumstances. Second, it serves as a basement membrane transplant (substrate) allowing the epithelium to grow over it. The use of amniotic membrane in the reconstruction of the ocular surface for various pathologic conditions relates to its function as a basement membrane substrate. And third, it expresses growth factors and cytokines that promote epithelialization, suppress inflammation and fibrosis, and inhibit angiogenesis. Amniotic membrane increases epithelialization by producing a number of growth factors, including b-FGF (fibroblastic growth factor) and HGF (hepatocyte growth factor).[7] It suppresses inflammation and fibrosis by down regulating the transforming growth factor-b (TGF-b) signaling system with the prevention of fibroblast activation into myofibroblasts.[8,9] Amniotic membrane also has antiangiogenic effects due to the expression of tissue inhibitors of metalloproteases and endostatin.[10]

MATERIALS AND METHODS

A prospective analysis of 40 eyes of 36 patients with mean age group of 32.5 years who underwent non-preserved amniotic membrane transplant between July 2016 to January 2018 were included in this study. Routine investigation, HIV and HbsAg status of the patients under study were screened. Donor consent was taken and status for HIV/HbsAg was checked before procurement of human placenta from elective caesarean section. Placenta was rinsed, cleaned and put in normal saline with gentamycin. With all pre-operative and precautionary measures separation of amnion from chorion was done by blunt dissection. After debridement of wound and removal of abnormal corneal epithelium, the conjunctiva was recessed to 4-5 mm from limbus. With the epithelium basement membrane surface up- multiple layers of non-preserved amniotic membrane were placed in ulceration site of non-healing corneal ulcers (12 eyes), ulceration due to dry eyes (12 eyes), surface epithelial defect in Steven Johnson Syndrome (8 eyes), which were further covered by another amniotic membrane layer and sutured to the edge of conjunctiva with 10-0 Ethilon. Single layer of non-preserved amniotic membrane was applied to cover epithelial defect due to alkali burn (8 eyes). Post operatively patients were given antibiotics, lubricants and anti-inflammatory/ immunosuppressive drugs wherever felt necessary. Sutures were removed on 31st day of transplant. Patients were evaluated for final visual outcome, corneal clarity, re epithelisation and degree of neovascularisation on 18th, 31st and 45th day. Patients were followed up for a mean period of 6 months. Failure of cases was diagnosed as reappearance of corneal haze, worsening of final visual acuity, secondary bacterial infection and non-healing of ulceration.

RESULTS

Out of 36 patients, 16 were males and 20 were females with mean age group of 32.5 years (table-1). 12 males out of them were suffering from ulcer due to dry eye. 8 females and 4 males were found to have non-healing corneal ulcer. All the 8 patients with Steven Johnson Syndrome were females and 4 females showed corneal injury due to alkali burn. 8 eyes (20%) of non-healing corneal ulcer showed resolution of epithelial defect and stabilization of neovascularisation earliest by 18th day and 4 eyes (10%)

initially showed good response but developed unrelated bacterial keratitis after 21st day of transplant. 8 eyes (20%) of corneal epithelial defect due to Steven Johnson syndrome showed healing with subsidence of acute inflammatory symptoms but 4 eyes (10%) of corneal epithelial defect due to Steven Johnson syndrome showed good response initially but developed unrelated bacterial keratitis after 21st day of transplant. 8 eyes (20%) of corneal epithelial defect due to Stevens Johnson syndrome showed healing with subsidence of acute inflammatory symptoms but 4 eyes (10%) failed due to bacterial super infection. 8 eyes (20%) with ulceration due to dry eye showed good response with re-epithelisation and stabilization of neovascularisation by 18th day of post-transplant and 4 eyes (10%) failed to show any response. Decrease in corneal haze and re epithelisation was seen in alkali burn in 4 eyes (10%). For the other eye (10%) of the same patient of alkali burn re-transplantation of amniotic membrane was done after 45 days. Preoperative visual acuity and postoperative visual acuity was compared table 2. It has been observed that the improvement in best corrected visual outcome following amniotic membrane transplant was observed in ulcer due to dry eye syndrome - 6/24 and 6/36 in 8 eyes and there was decrease in BCVA at least in 4 cases of non-healing corneal ulcer (HM+ from CF ½). BCVA in alkali burn patients was 4/60 in at least 1 patient. Out of 12 patients of Non-healing corneal ulcer 8 patients had improvement in BCVA (5/60, 4/60, 3/60 and 2/60) but 4 cases showed decrease in vision (HM+ from CF½). Maximum corneal clarity was seen in ulceration due to dry eye syndrome (8 eyes) and minimal improvement was seen in alkali burn table 3. Failure of transplantation was observed in 16 eyes (40%) - 4 dry eye syndromes, 4 Stevens Johnson syndromes, 4 non-healing corneal ulcers, 4 Alkali burn table 4. We observed a longer re-epithelisation time of 18 days as compared to preserved amniotic membrane transplant.

Sl. No.	Disease	Mean age	Male	Female
1	Non-healing corneal ulcer	30 yrs.	4	8
2	Ulcer due to dry eye syndrome	32 yrs.	12	0
3	Epithelial defect in Stevens Johnson Syndrome	35 yrs.	0	8
4	Alkali burn	33 yrs.	0	4
Total		**32.5 yrs.**	**8 (4.44%)**	**10 (55.6%)**

Table 1. Age and Sex Distribution

Sl. No.	Disease	Pre-transplant visual acuity	Post-transplant visual acuity on 31st day
1	Non-healing corneal ulcer	1.CF (1 mtr.) 2.CF (½ mtr.) 3.CF (½ mtr.) 4.CF (½ mtr.) 5.HM+ 6.HM+ 7.CF (1 mtr.) 8.CF (1 mtr.) 9.HM+ 10. CF (½ mtr.) 11.CF (1 mtr.) 12.HM+	3/60 2/60 HM+ HM+ 4/60 5/60 4/60 3/60 1/60 HM+ HM+ 2/60
2	Ulcer due to dry eye syndrome	1.6/60 2.4/60 3.2/60 4.4/60 5.6/60 6.6/36 7.6/36 8.5/60 9.4/60 10.6/24 11.6/60 12.3/60	6/24 2/60 6/60 2/60 6/60 6/36 6/24 6/36 6/60 6/18 6/24 6/36
3	Epithelial defect in Stevens Johnson Syndrome	1.3/60 2.2/60 3.4/60 4.2/60 5.4/60 6.5/60 7.3/60 8.HM+	6/60 2/60 4/60 5/60 4/60 3/60 6/60 2/60
4	Alkali burn	1.CF (1 mtr.) 2.CF (½ mtr.) 3.4/60 4.2/60	4/60 1/60 1/60 6/60

Table 2. Comparison of Visual Acuity

Sl. No	Diseases	Re-epithelisation	Stabilization of neovascularisation	Corneal clarity
1	Non-healing corneal ulcer	8 (20%)	8 (20%)	8 (20%)
2	Ulcer due to dry eye syndrome	8 (20%)	8 (20%)	8 (20%)
3	Epithelial defect in Stevens Johnson Syndrome	4 (10%)	4 (10%)	4 (10%)
4	Alkali burn	4 (10%)	4 (10%)	4 (10%)

Table 3. Results on 31st Day of Transplant

Sl. No	Disease	Success (No. of eyes)	Failed (No. of eyes)
1	Non-healing corneal ulcer	8 (20%)	4 (10%)
2	Ulcer due to dry eye syndrome	8 (20%)	4 (10%)
3	Epithelial defect in Stevens Johnson Syndrome	4 (10%)	4 (10%)
4	Alkali burn	4 (10%)	4 (10%)
Total		24 (60%)	16 (40%)

Table 4. Successful vs. Failed Cases

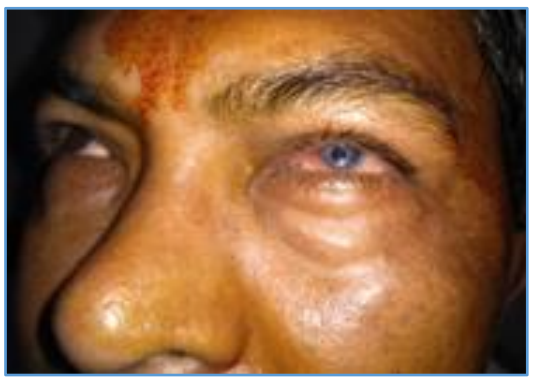

Figure 1. Alkali Burn

Figure 2. Severe Dry Eye

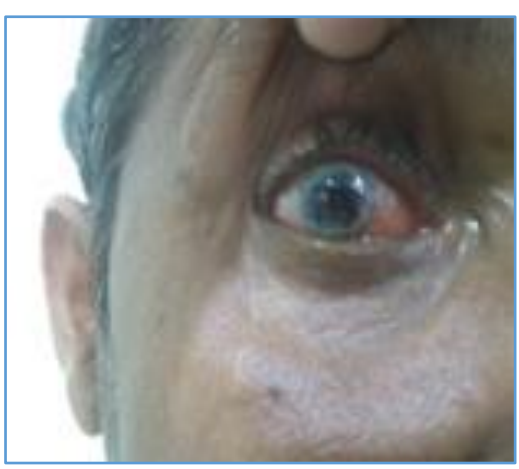

Figure 3. Non-Healing Corneal Ulcer

Figure 4 & 5. Placenta and Amniotic Membrane Collected from CS

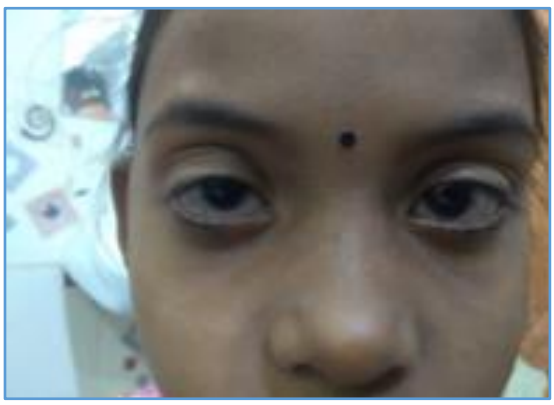

Figure 6. Postop Amniotic Membrane Transplant

DISCUSSION

Non-preserved Human Amniotic membrane is a safe and effective procedure in reconstruction of ocular surface disorders as compared to preserved amniotic membrane transplant.[11,3] 60% of our patients during clinical trial has shown re-epithelisation, corneal clarity, stabilization of neovascularisation and improvement in visual acuity which is comparable with published data (68%) with preserved membrane transplant.[3] The acute symptom of Stevens Johnson syndrome were relieved due to anti-inflammatory and barrier action of amniotic membrane. Deep corneal ulcers responded well to multilayer amniotic membrane transplant.[12] Retained ocular surface integrity and increase in corneal clarity is due to stem cells property of epithelial cells of Amniotic membrane.[11,8] 2 eye of Alkali burn which did not respond, had severe ocular surface defect leading to limbal stem cell deficiency which couldn't be compensated by Amniotic membrane graft alone and may need limbal allografts.[2,8] As compared to the published data by Taylor RJ, Wang MX *et al* invest. Ophthalmol.1998. regarding re-epithelisation time (10 days) with preserved Amniotic membrane transplant, we observed a longer period of 18 days.[13] Although the present study involved small no. of patients, a definite trend is evident where non-preserved amniotic membrane can be utilized in ocular surface reconstruction with less cost and equivalent success results. The major concern with the use of non-preserved amniotic membrane over preserved membrane is the risk of HIV infection despite seronegativity of the donor at the time of harvesting. Owing to the window period between infection and seroconversion, preserved amniotic membrane is released for use only if the donor is seronegative at the time of harvesting and at 6 months thereafter. None of our donors or patients was found seropositive for HIV, hepatitis B, or C the day before surgery or at 6 months postoperatively. Although rare, a risk exists that the donor could become infected shortly before harvesting, which could not be elicited in the serologic studies performed before surgery. Another concern about using non-preserved amniotic membrane is the risk of immune graft rejection. Human amniotic cells do not express HLA-A, B, C, or DR antigens on their surfaces.[14] On the other hand, expression of HLA-G antigen (major histocompatibility complex class Ib)

was found on amnion epithelium, mesenchymal cells, and fibroblasts.[15] If viable epithelial cells are present after cryopreservation as reported previously,[15] then there is at least a theoretical risk of immune graft rejection after transplantation of preserved amnion graft. On the other hand, human amniotic epithelial cells were reported to survive for 3 months after transplantation of non-preserved amniotic membrane.[16]

CONCLUSION

Reconstructing the lost ocular surface is one of the most serious challenges for an eye care professional. And when we talk about the availability of resources in our set up, it is better to quit than to face such challenges. Non-preserved human amniotic membrane transplant has definitely revolutionized and tamed the lost battle in reconstructing the ocular surface defect. AMT with non-preserved amniotic membrane promoted epithelial healing, reduced surface inflammation, increased patient comfort and decreased severity of vascularization and opacity in various surface disorders. In limbal stem cell deficiency owing to past injury to the cornea non-preserved amniotic membrane transplant has aided in the ocular surface reconstruction. It is a boon to those patients in our country where preservation facilities for amniotic membrane are not available and cost is a prime factor. Research on the use of non-preserved amniotic membrane in ocular surface disorder still needs more clinical trials, but our experience during this study has shown a definitive good result in terms of efficacy and cost which is comparable with that of preserved amniotic transplant.

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