Comparison of Efficacy of Storage of Amniotic Membrane at -20 and -80 Degrees Centigrade

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ABSTRACT
Objective: To compare the efficacy of storage of amniotic membrane at -20 and -80 degrees centigrade.
Study Design: Interventional quasi-experimental study.
Place and Duration of Study: Department of Ophthalmology, Unit 1, Dow University of Health Sciences and Civil Hospital, Karachi, from January 2009 to December 2010.
Methodology: Amniotic membrane was obtained from patients undergoing elective Caesarean section screened for HIV, Hepatitis B and C and gave informed consent. It was washed with solutions containing benzyl penicillin, streptomycin and amphotericin B and frozen at -20 degrees centigrade for up to 6 weeks or at -80 degrees centigrade for up to 6 months. Fisher's exact test was used to check significance. P-value less than 0.05 was considered as significant.
Results: Amniotic membrane was stored at -20 degrees centigrade on four occasions and was used in 25 (48.07%) patients. On 3 other occasions it was stored at -80 degrees centigrade and was successfully used in 27 (51.92%) patients. The association between different degrees of storage of graft and type of cases is insignificant (p = 0.99).
Conclusion: Compared to use of fresh amniotic membrane use of amniotic membrane stored at -20 and -80 degrees is safe and cost effective. It also excludes the chances of disease transmission. Although freezing at both temperatures is equally efficacious, freezing at -80 degrees centigrade can preserve the graft up to 6 months compared to -20 degrees centigrade which can be used only for 6 weeks.

Patients undergoing elective Caesarean section were selected for the study. Informed consent was taken and they were screened for Hepatitis B and C and HIV. Under full surgical preparation, with scrubbing, donning of gowns, caps and masks and surgical gloves the placenta was collected in a sterile kidney tray. The amnion and chorion were separated and the amnion was cut off at the umbilical cord and was taken in a separate sterile container. It was then washed in a solution containing Amphotericin B in a strength of 2.5 microgram/ml, Streptomycin (50 microgram/ml) and Benzyl Penicillin (50 microgram/ml).

Amniotic membrane was spread on sofratulle on the back of a sterile kidney tray. It was then cut into 5 x 5 cm pieces. Each piece was individually wrapped in sterile gauze. Ten to 15 pieces were prepared from each amniotic membrane and stored in a sterile clear container and stored at -20°C.

When preserved at -80°C the membrane was kept in DMSO (dimethyl sulphoxide) solution of concentration 4, 8, and 10% for 5 minutes each under a laminar flow hood. It was then spread on sofratulle on the back of the kidney tray and was cut into 5 x 5 cm pieces and stored in test tubes containing 10% DMSO. Approximately thirty pieces could be prepared from each amniotic membrane and stored at -80°C.

These pieces of amniotic membrane were used as ocular grafts. The amniotic membrane grafts were applied to the eye using 10/0 nylon sutures. Those pieces left after the period of preservation (6 weeks for -20 degree grafts and 6 months for -80 degree grafts) were discarded.

In the first 2 months of the study, the amnion was stored at -20°C in PWA's blood bank at Civil Hospital, Karachi. At this temperature it can be stored for 6 weeks. In the later part of the study it was stored at -80°C at the pathology laboratory of Sindh Institute of Urology and Transplantation where it could be stored for 6 months.

Data analysis was performed by using SPSS version 10. Percentages and frequencies were calculated for categorical variable. Fisher’s exact test was used to check the association of amniotic membrane at -20°C and -80°C. P < 0.05 was considered as significant.

**RESULTS**

A total of 52 patients were part of the study. Amniotic membrane obtained on four occasions was stored at -20°C and was used in 25 (48.07%) cases. In the later part of the study on 3 occasions it was stored at -80°C and was successfully used in 27 (51.92%) patients (Table I). Total number of patients was 52. There were 29 (55.76) cases of corneal ulcers, 18 (34.61%) cases of pterygium, 3 (5.76%) cases of burn and one (1.92%) each of Bowen’s disease and exposed lateral rectus.

**Table I: Storage of amniotic membrane.**

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Number of times amniotic membrane was taken</th>
<th>Number of patients</th>
</tr>
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<tbody>
<tr>
<td>-20°C</td>
<td>4 (16%)</td>
<td>25 (48.07%)</td>
</tr>
<tr>
<td>-80°C</td>
<td>3 (11.11%)</td>
<td>27 (51.92%)</td>
</tr>
</tbody>
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Categorical variables are presented as frequencies and percentages.

Out of the 18 cases of pterygium, 7 (38.8%) were primary and 11 (61.1%) recurrent. -20 degrees graft was used for 4 cases of primary and 7 cases of recurrent pterygium. Whereas -80 degrees graft was used for 3 cases of primary and 4 cases of recurrent pterygium. The association between different degrees of graft and types of cases is insignificant (p=0.999).

**DISCUSSION**

The use of amniotic membrane in ophthalmology started in 1940 and since then it has variously been used for treatment of persistent corneal defects, corneal perforation, conjunctival reconstruction following malignancy, burns, symblepharon in Stevens Johnson syndrome, limbal stem cell transplantation in severe ocular surface disease, dry eye, prevention of recurrence of pterygium, large leaking filtering blebs and preventing scar after photorefractive keratectomy.

When using autografts the problem of healing at the donor site can occur, which is not so when using amniotic membrane. Amniotic membrane stimulates re-epithelialization and facilitates migration of epithelial cells over it. It is not invaded by blood vessels, reduces fibrosis in healing tissue and unlike other allografts does not trigger immunological rejection. In addition it has antimicrobial effects. The method of preparation affects the angiogenic factor profile of the amniotic membrane.

Although the use of amniotic membrane in ocular surface disorders is well established, its application in clinical practice is not widespread, the reason for this being the lack of ready availability. Fresh amniotic graft carries the risk of transmission of infections including HIV and hepatitis etc. Preservation allows for screening of donors and preparation costs are considerably reduced. Upto 30 samples can be prepared from one placenta.

In a study conducted to assess influence of storage time on sterility, histological and biological properties of cryopreserved amniotic membrane where membranes were discarded.
from different donors were stored in cell culture media containing 50% glycerol for different time periods from 4 to 24 months at -80°C, none of the examined samples showed bacterial or fungal contamination.\footnote{Thomasen H, Pauklin M, Noelle B, Geerling G, Vetter J, Osterhouts D. Amniotic membrane preservation: a randomized controlled trial comparing radiation sterilization to cryopreservation. JAMA 2014; 312:2320-6.}

In the current study amniotic membrane stored at -20°C and at -80°C were variously used for different indications. In all cases, there was good graft incorporation and in none of the cases there was any infection or marked inflammatory response. The grafts were stored at -20°C in the Blood Bank of Civil Hospital, Karachi and were readily available. The samples preserved at -80°C required storage at a research laboratory in Sindh Institute of Urology and Transplantation. Although the graft could be safely preserved for a long period, these had to be brought to the theatre at a pre-arranged time.


Preservation of amniotic membrane at -80°C has the advantage of availability for 6 months. However, the facility of storage is only available in research laboratories. Moreover, it requires laminar flow hood and dimethyl sulfoxide solution and the graft pieces are stored in test tubes. These pieces are not available in emergency situations such as burns and corneal ulcers where grafts may be needed at short notice.

Most blood banks at major hospitals have the facility of refrigeration of blood products at -20°C temperature. At this temperature the graft can be stored safely for use for 6 weeks. Since the blood banks are within the hospital premises there is ready availability of graft material even in cases of emergency. The preparation for storage is also much simpler and does not require laminar flow hood and DMSO.

In the present study there was no issue regarding graft procurement. After informed consent all donor mothers agreed for the use of placenta for harvesting the graft. Likewise, once the purpose and implications of the study had been explained none of the potential recipients of the study opted out. The association between different degrees of graft and type of cases was found to be statistically insignificant.

It appears that the case for use of amniotic membrane in ocular surface disorders is well established although the preferred method of preservation remains debatable. In developed countries tissue banks for amniotic membranes are well established as are in some centres of Pakistan such as Shifa Trust Hospital in Islamabad and the Aga Khan Hospital in Karachi. However, given the size of clinical practice in ophthalmology many such tissue banks are required in the country.

**CONCLUSION**

Storage of amniotic membrane at -20°C is as safe and efficacious as that at -80°C, although the 6 weeks period of storage of the former method is much shorter than the 6 months availability of the latter method. Ready availability of storage at -20°C at major hospitals makes it a more attractive method of storage in local setting.

**REFERENCES**


