AMNIOTIC MEMBRANE APPLICATIONS – OUR EXPERIENCE

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The authors of the study declare that no conflict of interest exists in the compilation, theme and subsequent publication of this professional communication, and that it is not supported by any pharmaceuticals company.

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SUMMARY

Introduction: Amniotic membrane is the innermost part of the fetal and packaging for its exceptional qualities likes to be used in treating many ocular pathologies. Amniotic membrane has improved the ability to treat ocular surface disease. It has unique features like support conjunctival and corneal epithelialization.

Material and methods: Retrospective analysis of group patients who underwent amniotic membrane transplantation at the Department of Ophthalmology Faculty of Medicine and UN Bratislava in 2013–2015. We evaluated indications amniotic membrane transplantation, the percentage, the number of transplants and the number of failures and retransplantation of the membrane.

Results: In group of 71 patients (amniotic membrane covering defects of conjunctiva and cornea) male patients formed a slight predominance of males in the number of patients a slightly larger preponderance in 38 women (53.5 %) – 52 surgeries (59.09 %) and 33 male (46.5 %) in 36 interventions (40.91 %). The left eye was affected in 40 interventions (45.45 %), 48 interventions were on the right eye (54.54 %). The most common cause application of 30.68 % in 27 eyes was corneal ulcer, bullous keratopathy followed by the 11.36 % in 10 eyes, and the ulcer herpetic keratitis in 9.10 % in 8 eyes. Injury or vulnus penetrans 6.82 % in 6 eyes, ulcers caused by paresis n. facialis 6.82 % in 6 eyes and sicca syndrome 5.68 % in 5 eyes.
In 2015 we applied amniotic membrane covering the defect of eyelids after trauma in one patient.

Conclusion: Amniotic membrane is the appropriate treatment in a number of diseases of ocular surface when conservative methods of treatment fail. In corneal application can prevent the execution of more aggressive treatment, such as keratoplasty, or to soothe inflammation and keratoplasty is not performed as emergent, but elective.

Key words: amniotic membrane transplantation, eye diseases

Čes. a slov. Oftal., 72, 2016, No. 6, p. 204–208

INTRODUCTION

In ocular surgery several properties and mechanisms of the effect of amniotic membrane have been described, which make it a suitable substrate for transplantation in this branch of surgery. The mechanisms of the effect are derived especially from the composition of the membrane, and not all can be applied to a fresh or preserved membrane. Preserved membranes are considered inert, and therefore do not contain any viable cells. Such a membrane has a limited capacity to influence the healing of a wound by means of a spectrum of growth factors and cytokines [4].

Transplantation of a human amniotic membrane is used clinically especially in the reconstruction of the corneal and conjunctival surface, where it supports healing of the epithelium, alleviates inflammatory reaction and the formation of scars. The similarity of the amniotic membrane to limbal stems has indicated its use as a substrate for ex vivo expansion of limbal stem cells and their subsequent transplantation, together with the membrane. This method is widely used in practice today. The membrane is also used in glaucoma surgery and oculoplastic procedures. The numerous clinical indications for the use of the amniotic membrane include corneal ulcers, pterygia, chemical and thermal burns, glaucoma surgery, symblepharon and others [11, 12].

The amniotic membrane serves as a foundation membrane which facilitates the migration of epithelial cells, strengthens the adhesion of epithelial cells, supports epithelial differentiation and averts apoptosis of the epithelium. By means of an unknown mechanism it also improves corneal sensitivity and stability of tears [1, 2, 4, 5].

It produces various growth factors, which stimulate epithelisation, and some authors are also of the opinion that it supports the expansion and extends the life of epithelial progenitor cells in vivo [7].

Epithelial cells of the membrane produce brain natriuretic peptide (BNP), a corticotropin releasing hormone (CRH) which assists in increasing cell proliferation and plays a role in the calcium metabolism [2]. It has been determined that a cryoconserved amniotic membrane exprimates mRNA for epidermal growth factor (EGF), hepatocyte growth factor (HGF), keratocyte growth factor (KGF), as well as the receptors for these factors [6].

Proteoglycans in the matrix of the membrane are important for cell proliferation and differentiation by binding growth factors, fulfilling an important role in remodelling processes [10].

By means of these mechanisms amniotic membrane may accelerate healing of the epithelium. Laminin isoforms, which are located in the basal membrane, facilitate the adhesion and expansion of corneal epithelial cells. This capability is also applied in the treatment of partial defect of limbal stem cells.

Amniotic membrane may also be used as a covering contact lens, which enables epithelisation beneath its surface. It

has good permeability, and thereby provides sufficient oxygenation for epithelial cells in comparison with synthetic materials [2].

The practically unlimited availability and low costs for acquisition and processing make amniotic membrane an important biomaterial in medicine. The path from obtaining a donor placenta to transplantation of an amniotic membrane is complex. Before clinical application it must undergo screening for various pathologies and the approved method of processing, which incorporates de-epithelisation, sterilisation and conservation.

Several methods of these procedures of processing exist, and each method of preparation has a different effect on the physical and biological properties of the amniotic membrane. As a result it is difficult to determine a standardised method of preparation of the membrane.

Upon each operation in which transplantation of organs or tissues takes place, there is a certain risk of transmission of disease. For this reason the safety measures and protocols used upon organ transplantation relate also to transplantation of amniotic membrane in ocular surgery [3].

Upon vaginal birth, amniotic membrane is frequently contaminated with vaginal flora, as a result of which the membrane is obtained under sterile conditions from donors giving birth by means of elective caesarian section.

The potential donor must undergo screening for pathologies and social risk factors which would represent a risk for the transmission of infection. In the anamnesis we inquire with regard to risk sexual behaviour, blood transfusion, intravenous drug abuse and tattooing. After informed consent has been obtained, as close as possible to the day of birth the blood of the donor is examined for HIV-1, 2, hepatitis B, C (HBsAg, anti-HCV), human T-cell lymphotropic virus (HLTV), syphilis, cytomegalovirus (CMV), Toxoplasma gondii and tuberculosis (TBC).

In the Central Tissue Bank in Bratislava cryoconserved amniotic membrane is prepared, stored at a temperature of -70°C in a cryoprotectant medium, which contains 10 % dimethyl sulfoxide (DMSO). Before use the membrane is always rinsed three times in a suitable medium in order to remove residues of the cryoprotectant solution.

MATERIAL AND METHODS

Analysis of patients who underwent transplantation of an amniotic membrane at the Department of Ophthalmology, Faculty of Medicine, Comenius University and University Hospital Bratislava in the period of 2013 – 2015. We evaluated indications for transplantation of an amniotic membrane, its success rate, the number of failures of transplantations and number of re-transplantations of a membrane. We evaluated the fundamental demographic characteristics of the cohort.

RESULTS

In a cohort of 71 patients for whom application of an amniotic membrane was indicated in order to cover a defect on the ocular surface (conjunctiva, cornea), the average age was 63.7 years, in which the youngest patient was aged 22 years and the oldest patient of the cohort underwent surgery at the age of 87 years. The median age of the cohort was 64 years and the modus, i.e. the most frequently occurring age, was 70, 71 and 73 years. There was a slight predominance of women over men in the number of patients, and a somewhat larger predominance in the number of procedures – 38 women (53.5%) with 52 procedures (59.10%) and 33 men (46.5%) with 36 procedures (40.9%). 40 procedures (45.45 %) were performed on the left eye, on the right eye 48 procedures (54.54 %).

In our cohort in 2013 we applied an amniotic membrane in 25 cases, with more men than women undergoing surgery, in 2014 a membrane was applied in 41 cases, with a predominance of women, and in 2015 in 22 cases, with a slight predominance of women (Table 1).

In 2015 we applied an amniotic membrane in one case in order to cover a defect of the eyelids in a patient – homeless person following injury (fig. 1 – 4).

The most common indication was corneal ulcer of unknown origin in 30.68 % of cases (27 eyes), followed by bullous keratopathy in 11.36% (10 eyes) and ulcers due to herpetic keratitis in 9.10% (8 eyes). Post-traumatic conditions (burns) were treated in 6.82% (6 eyes), ulcers due to paresis n. facialis in 6.82% (6 eyes) and sicca syndrome in 5.68% (5 eyes). Ulcers following excision of basalioma 2.27% (2 eyes), post-radiation keratitis 2.27% (2 eyes), dry stain 2.27 (2 eyes), ulcer caused by ectropion 1.14% (1 eye), trichiasis 2.27% (2 eyes) and other ulcers (following perforating keratoplasty, re-transplantation of amniotic membrane) 19.32% (17 eyes).

In one patient we applied an amniotic membrane to cover a defect of the eyelids following injury.

The most common indication for application of an amniotic membrane was the group of patients with corneal ulcers, in which we did not know or did not succeed in determining the cause. In this indication transplantation was performed 27 times, which represents 30.68% of all cases.

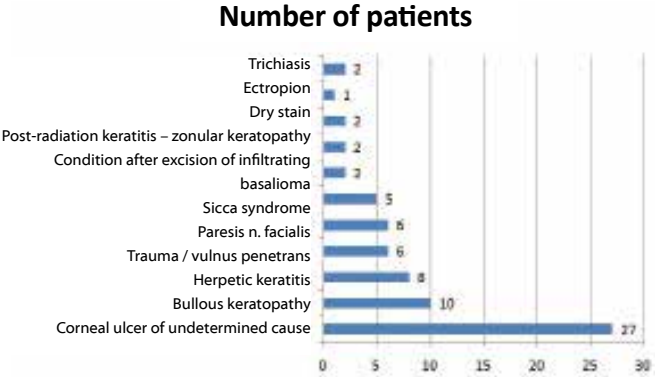
The second most common cause of transplantation of an amniotic membrane was bullous keratopathy with the formation of ulcers or other superficial defects of the cornea, in which a membrane was applied in 10 cases, representing 11.36% of all cases.

Graphs 1 and 2 present the different causes for which application of an amniotic membrane was indicated.

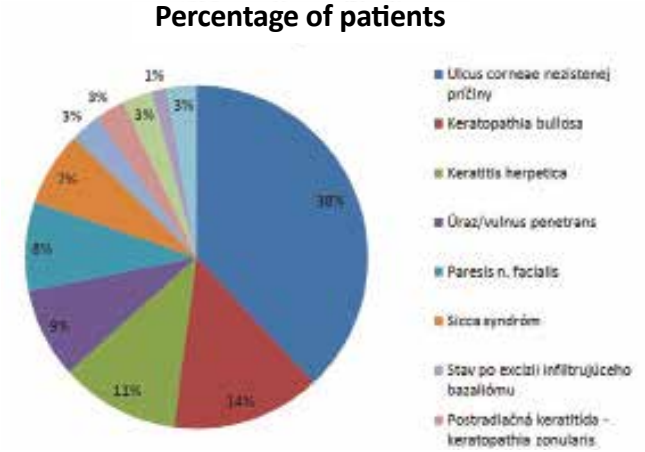
Average central visual acuity (CVA) in 31 patients was better than 0.1 after surgery, of these 2 patients had vision of 1.0 and 4 patients 0.8. In 20 patients CVA was less than 0.1 (counting fingers in front of eye) in 14 patients CVA was stated as hand movement, in 4 patients light sense with localisation, and in 2 without light sense.

Of the total number of patients, failure occurred in 17 eyes (19.32%), in which the criterion for failure was considered to be persistent corneal defect and/or necessity of re-transplantation of amniotic membrane, keratoplasty or evisceration of the eyeball.

Tarsorrhaphy was performed on 9 patients (18 %) in the observed cohort. In all patients healing took place following the suturing of an amniotic membrane with subsequent tarsorr-



Graph 1 Indications for application of amniotic membrane



Graph 2 Percentage distribution of indications for application of amniotic membrane

haphy. In 2 patients a recurrent defect occurred, as a result of which tarsorrhaphy together with an amniotic membrane was performed a total of 3 times on one patient.

In our cohort 4 patients had a transplanted amniotic membrane following keratoplasty. The procedure was successful in 3 out of 4 eyes (75 %).

In 1 patient (25%) a persistent defect in connection with Fuchs' dystrophy was not healed within the observed period.

DISCUSSION

In their study Letko et al. from the Harvard Medical School in Boston compared the effectiveness of treatment with an amniotic membrane on 30 patients with epithelial defects caused by sicca syndrome, infectious ulcers, radiation keratopathy, pemphigus scarring, bullous keratopathy, chemical burns, herpetic keratitis, atopic keratoconjunctivitis and others. The patients had an average age of 55.3 years. Average CVA of the patients after surgery was 0.05 [9].

After the first application of a membrane, 21 eyes (70%) healed and in 9 cases (30%) the defect persisted after the first application of a membrane. Recurrence was recorded in 6 cases (29%) out of 21. The average time of the first recurrence of the defect was 5.2 weeks (36.7 days). During the observation transplantation of an amniotic membrane was repeated on 5 patients. In 16 patients tarsorrhaphy was also performed simultaneously upon the first transplantation, in 12 patients (75%) the defect was healed, and in 4 of these (33%) recurrence occurred. Similar results were recorded also in patients without tarsorrhaphy, in which the defect was successfully healed in 9 patients (64 %) out of 14, and in 2 patients (22 %) out of 9 recurrence occurred [8].

Fig. 1 Patient following head and face injury after primary treatment and suture in the superciliary area, with extensive skin defect in the region of the eyelids of the right eye.

FIg. 2 Application of amniotic membrane within the scope of superficial defect of the upper eyelid

FIg.. 3 Course of healing under application of greased gauze

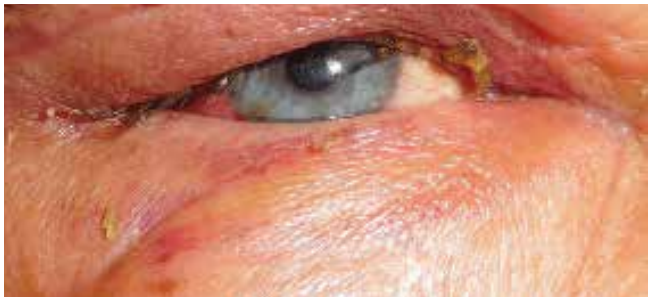


FIg.. 4 Condition after 2 months of healing

This cohort of patients had a similar composition to that of the patients in the observed cohort over the course of 3 years at the Department of Ophthalmology at the Faculty of Medicine, Comenius University and University Hospital in Bratislava, and is thus suitable for comparison of results.

In the patients we observed, the average age was 63.72 years, with a predominance of women, and the average CVA of the patients after surgery was more than 0.1 in 31 patients, in 20 patients counting fingers in front of the eye, in 14 patients CVA was stated as hand movement, in 4 patients light sense and in 2 patients without light sense.

Of the total number of patients, 71 eyes (80.68%) were successfully healed by the application of an amniotic membrane. Failure of treatment occurred in 17 eyes (19.32%), in which the criterion for failure was considered to be persistent corneal defects and the necessity of re-transplantation of the amniotic membrane, keratoplasty or evisceration of the eyeball, of which 5 eyes required subsequent tarsorrhaphy. Following transplantation of an amniotic membrane, provided that their overall condition of health so permits, the patients are being regularly monitored.

In their study, Pinnita Prabhasawat et al. from Mahidol University in Bangkok also evaluated the effectiveness of transplantation of an amniotic membrane in 28 patients with corneal defects or without thinning of the cornea and with corneal perforations, also with similar causes of origin as in the patients in our observed cohort. The average age of the patients was 48.7 years. The success rate of use of amniotic membrane was 82.1 %, in which the eyes of 23 out of 28 patients were healed. Failure of transplantation occurred in 5 patients (17.9 %) with unnoticed active infection (occult fungal ulceration and geographic herpetic ulcer), neurotrophic keratopathy (2 patients) and corneal perforation. The membrane had to be transplanted repeatedly in 4 cases of failure, and in 4 cases (17.4% of successful cases) due to recurrence of the pathology due to abnormalities of the eyelids, post-radiation and neurotrophic keratopathy. In certain cases the operating surgeons used tarsorrhaphy as a supplementary procedure, but this was not evaluated in the study. At the same time the authors compared the speed of re-epithelisation upon the use of one and more layers of an amniotic membrane. The result was significantly higher speed of re-epithelisation upon multilayer grafts, which the authors connect with the larger quantity of matrices, providing a larger quantity of growth factors [13].

This study also demonstrated the significance of the use of an amniotic transplant, and in fact the observed values differed absolutely minimally. The success rate in this study was 82.1% and in our cohort 84.1%. Failure occurred in 17.9% as against 15.9% in our patients, and recurrence was 17.4% as against 15.15 %.

If we wish to compare the causes of failure of treatment in the observed patients with the study, in our patients failure occurred most frequently in the case of ulcers of unknown origin, in a total of 3 patients (42.8 %), followed by herpetic keratitis in 2 patients (28.6 %), and in one case upon hereditary dystrophy and once in treatment of dry stain – keratitis (14.3 % each). To a certain extent the reasons overlap, because neurotrophic keratitis or corneal perforation in the study may occur as a consequence of herpetic keratitis in our cohort, or ulcer of unknown origin may lead to perforation.

Upon a comparison of the causes of recurrence, in our patients recurrence occurred upon sicca syndrome, paresis n. facialis, post-radiation keratitis, herpetic keratitis, bullous keratophathy, hereditary dystrophy and ulcer of unknown origin. Again the causes are comparable. In addition to identical post-radiation keratitis, abnormalities of the eyelids may be connected with sicca syndrome and paresis n. facialis, whilst bullous keratopathy may be caused by a large number of ocular pathologies.

Seitz et al. from Saarland University in Germany conducted a study in which they examined the success of treatment of corneal epithelial defects following perforating keratoplasty with the aid of transplantation of an amniotic membrane, and their recurrence. Amniotic membrane can be used in patients with persistent epithelial defects following previous keratoplasty in order to protect a corneal transplant, for prevention of more invasive procedures such as re-transplantation, which increases the risk of immunological rejection of the transplant.

Success, i.e. complete epithelisation was achieved in 16 eyes (70%) and was indirectly proportional to the number of previous keratoplasty procedures on the given eye. Of 16 successfully treated eyes, 7 eyes (44%) manifested at least one recurrence of the defect.

The authors found an interesting correlation between recurrence and the time interval between transplantation of the membrane and previous keratoplasty. No recurrence was observed in cases when the interval was short (1-7 months), or conversely very long (79 - 120 months). The majority of recurrences were between 10 and 43 months [14].

In our cohort 4 patients had a transplanted amniotic membrane following keratoplasty. Success was achieved in 3 (75%) out of 4 eyes. Recurrence was recorded in 1 (33%) of 3 successfully treated eyes in a patient following penetrating injury, which however was no longer recorded after repeated transplantation of an amniotic membrane. The patient with recurrence underwent repeated keratoplasty and the time between keratoplasty and transplantation of the membrane was 5 months.

In 1 patient recurrence did not occur despite repeated keratoplasty. The interval between keratoplasty and application of the membrane in this patient was 30 months.

In 1 patient (25%) healing of a persistent defect in connection with Fuchs' dystrophy was not achieved within the observed period, and in 1 patient the condition was good within the observed period, but in 2015 rejection of the transplant occurred in this patient.

Our results are comparable with the study by Seitz, which relates to the success of use in this indication, with a slight deviation in recurrence. The correlation between the number of previous keratoplasty procedures and recurrence is ambiguous in our cohort. Similarly the interval between keratoplasty and transplantation of a membrane was not confirmed, which may however have been caused by the small number of observed patients meeting the criteria during the

course of the observed years.

We may attribute the slight deviations between the results of the observed cohort and the above-presented studies to the different surgical technique, various indications for application and also to their varying degree of severity and scope.

CONCLUSION

The role of amniotic membrane in the treatment of pathologies of the ocular surface is indisputable. The main advantages of amniotic membrane include the following: stimulation of re-epithelisation of the ocular surface, prevention of scarring, neovascularisation, suppression of inflammatory reaction. It does not trigger immunological reactions, enables migration of cells over its surface as well as their differentiation, forms a mechanical barrier for bacteria and protects the afflicted tissue, enables full growth of nerve endings and the conjunctiva, and thereby restores its sensitivity.

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