Amniotic membrane transplantation for ocular surface burns

Transplante de membrana amniótica em queimaduras da superfície ocular

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ABSTRACT

Objective: To analyze the morphological and functional long-term outcomes of amniotic membrane transplantation after ocular surface chemical burns.

Methods: This prospective study analyzed 7 patients who suffered from severe ocular surface burn and underwent amniotic membrane transplantation from 2015 to 2020 in *Hospital de Clínicas - Universidade Federal do Paraná*.

Results: Out of the seven patients, six (85.7%) suffered unilateral burn and one (14.3%) suffered bilateral burn. Five of them had alkali burns (71.4%), one had acid burn (14.3%) and one suffered gunpowder fireworks burn (14.3%). Mean age was 29.4 years (±standard deviation 13.3, range 14.0 to 47.0 years). Mean visual acuity at first presentation was 1.83±0.79 logMAR (0.015 decimal) and mean VA after a follow-up of 1 year was 0.85±0.70 logMAR (0.141 decimal). The visual acuity significantly improved from 1.83±0.79 to 0.85±0.70 logMAR ($p<0.05$).

Conclusion: Amniotic membrane transplantation is an effective adjunctive treatment in the management of ocular surface chemical burns with potential to improve the final vision outcome.

RESUMO

Objetivo: Analisar os resultados morfológicos e funcionais a longo prazo do transplante de membrana amniótica após queimaduras químicas da superfície ocular.

Métodos: Foi realizado um estudo prospectivo com análise de sete pacientes que sofreram queimaduras graves da superfície ocular e foram submetidos a transplante de membrana amniótica no período de 2015 a 2020 no Hospital de Clínicas da Universidade Federal do Paraná.

Resultados: Dos sete pacientes, seis (85,7%) sofreram queimadura unilateral e um (14,3%) sofreu queimadura bilateral. Cinco deles sofreram queimaduras por álcali (71,4%), um por ácido (14,3%) e um por pólvora de fogo de artifício (14,3%). A média de idade foi de 29,4 anos (±desvio-padrão de 13,3, intervalo de 14,0 a 47,0 anos). A acuidade visual média na primeira apresentação foi de 1,83±0,79 logMAR (0,015 decimal) e, após 1 ano de seguimento, foi de 0,85±0,70 logMAR (0,141 decimal). A acuidade visual melhorou significativamente, de 1,83±0,79 para 0,85±0,70 logMAR ($p<0,05$).

Conclusão: O transplante de membrana amniótica é um tratamento adjuvante eficaz no manejo de queimaduras químicas da superfície ocular com potencial para melhorar a visão final.

INTRODUCTION

Chemical burns are considered ocular emergencies that require immediate assessment and intensive care.[1] Each year, approximately 2 million cases of monocular blindness result from trauma and corneal ulceration worldwide.[2] Chemical burns account for 11.5%[3] to 22.1%[4] of these injuries, with alkali or acid exposure being the leading causes.[2]

Immediate effects of ocular surface burns include irreversible damage to cornea and conjunctiva. This injury to the outer layers of the eye exposes the corneal collagen bundles to chemical denaturation, resulting in varying levels of corneal opacity. Ischemia affects the blood vessels surrounding the cornea, compromising tissue viability.[2] The damage to the ocular surface can lead to chronic disorders such as limbal insufficiency, dry eye, symblepharon formation, fornix shortening, persistent inflammation, persistent epithelial defects, chronic pain, and compromised visual acuity (VA).[5] In the case of firework-related burns, a review showed that most injuries were mild and temporary, with high incidence rates for corneal abrasions (42.2%) and globe contusions (25.9%).[6]

Eye burns need immediate treatment and represent a risk of visual loss due to immediate injuries and subsequent sequelae. In the case of chemical burns, the initial and crucial step is to wash off the chemicals, which can both treat and prevent one of the most undesirable consequences, limbal deficiency, along with other complications.[5] Although not always available, the ocular surface's pH can be assessed using a urinary pH strip, and irrigation should continue until the pH normalizes to 7. Any remaining particles can be removed from the ocular surface using a moist cotton tip. The prognosis is closely related to the effectiveness of immediate treatment.[1]

The treatment during the acute phase depends on the findings on the examination. The main objectives are to promote re-epithelialization, reduce inflammation, prevent infection, prevent further epithelial and stromal rupture, and minimize long-term complications.[1] Immediate treatment involves a combination of antibiotics, preservative-free lubricants and steroids. Additionally, topical treatment may include cycloplegic drops to alleviate pain, ascorbate drops for collagen synthesis and wound healing, and citrate drops to reduce calcium chelation required by inflammatory polymorphonuclear cells.[1,7] In some cases, tetracyclines can aid in preserving corneal tissue by inhibiting matrix metalloproteinase activity.[2,8]

Topical corticosteroids play a crucial role in reducing inflammatory cell infiltration and stabilizing neutrophil cytoplasmic and lysosomal membranes. However, they also inhibit re-epithelialization and collagen synthesis. In alkaline burns, topical steroids should not be used for more than 10 to 14 days, as they increase the risk of corneal perforation. However, this concern primarily applies to severe lesions with persistent epithelial defects. If the epithelium has already closed, corticosteroids can be safely used beyond 7 to 10 days.[1,5]

The treatment involving amniotic membrane (AM) consists of its application to either a specific area or the entire affected ocular surface.[2] It is widely believed that, during the early stages, AM contributes to initial surface healing, leukocyte infiltration, and stem cell formation.[9] Acting as a basement membrane, AM stimulates epithelialization and facilitates cell migration. Moreover, amniotic cells secrete numerous growth factors that play a significant role in promoting corneal epithelial healing.[10]

In this prospective study, we conducted an analysis of the long-term morphological and functional outcomes following AM transplantation in cases of ocular chemical burns.

METHODS

The present study received approval from the Research Ethics Committee of *Hospital de Clínicas* of the *Universidade Federal do Paraná*. All participants provided informed consent and had their data collected and stored in accordance with ethical principles of privacy and confidentiality.

This prospective study analyzed a total of seven patients who suffered from severe ocular surface burns and were referred to the hospital from 2015 to 2020. All seven patients underwent AM transplantation with the goal of restoring the ocular surface and VA.

Visual acuity assessment was performed for all patients using Snellen's original test, with conversions to decimal and logMAR scales for statistical analysis. Lower VAs were classified as follows: counting fingers, 1/100 (logMAR 2); hand motions, 1/200 (logMAR 2.3); light perception, 1/666 (logMAR 2.8) and amaurosis, 0 (logMAR 3).

The AM was obtained from elective and term cesarean deliveries, with the consent of the parturient and her companion, at the same hospital where the patients with ocular surface burns were referred to. Donors underwent laboratory analysis for HIV, hepatitis types B, and C and syphilis. These serologies were reconfirmed analyzing umbilical cord blood after delivery. Positive results in any of the serologies were exclusion criteria for using the AM.

Placental preparation was conducted in the operating room under sterile conditions. The placenta was immersed in a diluted solution containing gentamicin antibiotic and thoroughly washed with 0.9% saline solution. The AM was then carefully separated from the chorion through blunt dissection and placed on sterile nitrocellulose filter paper, with the epithelium facing upwards. Pieces of approximately 10 x 5 cm were cut separately. The small AM samples were stored in a sterile manner at -80° Celsius in a 1:1 ratio solution of glycerol and Dulbecco's Modified Eagle Medium (Low Glucose - 1.0g/L) until they were used or discarded after 6 months.

All surgeries were performed by ophthalmologists. The surgical technique involved fixing the membrane to the ocular surface with 10-0 nylon sutures on the cornea and 8-0 vicryl sutures on the conjunctiva, with simple and/or continuous stitches. In all cases of this study, the membrane was placed on the ocular surface with the epithelium facing upwards. The vicryl sutures were removed 2 to 3 weeks after surgery, and the nylon suture over the cornea was removed around 2 to 4 months after complete absorption of the membrane.

Postoperatively, a combination of topical corticosteroids and antibiotics was administered to reduce the inflammatory process and prevent secondary infections. To relieve pain, discomfort, and increase membrane adhesion to the ocular surface, a therapeutic contact lens was placed after AM transplantation. All participants were followed up for a minimum of 1 year.

Statistical analysis was performed using Microsoft Excel 2000 and Graphpad Prism (Graphpad Prism for Windows 5.03). Spearman's correlation was used for non-parametric data, while the Mann-Whitney U test was used for non-parametric data with nominal components. Odds ratios (ORs) were calculated for variables related to success, along with their 95% confidence intervals (CIs). VA data were converted to the logMAR scale, and a Wilcoxon signed-rank test was applied to analyze the relationship between VA before and after the intervention. A *p*-value < 0.05 was considered statistically significant.

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RESULTS

Of the seven patients included in the analysis, four were male (57.1%) and three were female (42.9%). Among them, six patients (85.7%) had suffered a unilateral burn, with four in the left eye and two in the right eye, while one patient (14.3%) had a bilateral burn, but the AMT procedure was performed only in the left eye. Therefore, seven eyes were included in the study. Five patients (71.4%) had alkali burns, one had an acid burn (14.3%), and one had a burn from fireworks gunpowder (14.3%). The mean time from burn to AMT was 113.4 days (± standard deviation [SD] 98.01, range 6.0 to 300.0 days). The mean age of the participants was 29.4 years (±SD 13.3, range 14.0 to 47.0 years).

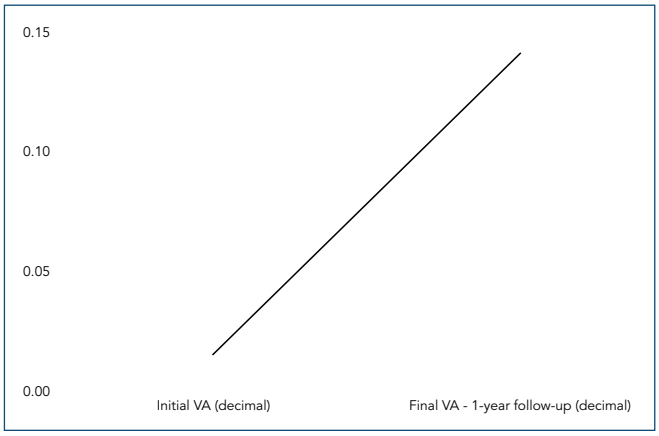
At presentation, two eyes were classified as Roper-Hall III (28.6%) and five eyes were classified as Roper-Hall IV (71.4%). Among the eyes, four (57.2%) required two or more additional procedures, and three of these eyes were classified as Roper Hall-IV (75%). Specifically, two eyes required two procedures (28.6%), one eye required three procedures (14.3%), and one eye required four procedures (14.3%). The additional procedures performed, in addition to AMT, included autologous and heterologous limbus transplantation, symblepharon removal, fornix reconstruction and granuloma excision.

Six months after the procedure, five eyes (71.4%) developed limbal stem cell deficiency, three eyes had corneal opacification (42.9%), and one eye had inflammation (14.3%). Prior to the AMT procedure, five eyes (71.4%) had symblepharon, and four eyes (57.2%) still had symblepharon after 6 months. No severe complications related to the AMT procedure were observed.

The mean VA at the initial presentation was 1.83±0.79 logMAR (0.015 decimal), while the mean VA at a 1-year follow-up was 0.85±0.70 logMAR (0.141 decimal). A final VA of 0.5 logMAR or better (0.3 decimal) was achieved in three eyes (42.8%). Four eyes (54.1%) ended up with a VA of 0.7 logMAR (0.2 decimal) or worse, and two eyes (28.6%) had a final VA of 1.0 logMAR (0.1 decimal) or worse. All eyes with a final VA of 0.7 logMAR (0.2 decimal) or worse were classified as grade IV Roper-Hall. Overall, the VA significantly improved from 1.83 ± 0.79 to 0.85 ± 0.70 logMAR ($p<0.05$) in the entire study population (Figure 1). The grade of ocular burn showed significant correlation with the VA at presentation (Spearman's correlation $p=0.034$), indicating that patients with less severe ocular burns, according to the Roper-Hall scale, had better initial VA.

DISCUSSION

The use of biological tissues, such as the human AM, has been employed in the repair and reduction of inflammation in patients with severe corneal and conjunctival injuries. The AM has been used in surgery since the beginning of the last century, although its use



VA: visual acuity.

Figure 1. Line graph showing significant visual acuity improvement (p<0.05).

in ophthalmology is relatively recent.[11] In general, AMT objectives in severe burns are rarely correlated to VA improvement.[2] However, in the present study, we observed a statistically significant improvement in VA (p<0.05), with three eyes (42.8%) achieving a final VA of 0.5 logMAR or better (0.3 decimal).

Complications can be expected in cases of ocular burns,[2] but it is important to note that most of these complications arise from the underlying disease rather than the AMT procedure itself. Postoperative infection is a potential complication of AMT, although its incidence is low (<1.6%).[12] In our study, none of the cases presented this complication during the 1-year follow-up.

The stage of an ocular burn plays a significant role in predicting resolution and outcomes. The extent of surviving limbal tissue is an important prognostic factor. The extent of corneal limbus and conjunctival involvement can be used to estimate the probability of corneal epithelialization failure, which is a critical event in wound healing.[2] The Roper-Hall classification system was developed in 1960, first by Ballen,[13] and later modified by Roper-Hall.[14] Burns were graded from I (mild) to IV (severe) according to the degree of corneal opacity, limbal ischemia and epithelial involvement at presentation.[2] In our study, all our patients were classified as Roper-Hall grade III or IV, with grade IV being the most prevalent. The classification indicates extensive corneal damage and limbal ischemia, highlighting the severity of the burns in our cases.

In our study, three patients underwent limbal stem cell transplantation in addition to AMT. One patient required penetrating keratoplasty, and another patient underwent fornix reconstruction with AM. It is important to note that only two patients underwent AMT once. Despite the variation in treatment approaches based on individual cases, the overall results showed positive outcomes in terms of VA evaluated one year after the last AMT, which differs from the majority of studies in the literature. A review published in 2015 demonstrated that VA can improve in patients with moderate burns (p=0.007), but there was no significant improvement in patients with severe burns, when compared to the control group (p=0.79).[2] A crucial consideration is the need for long-term and through outpatient follow-up for patients with eye burns. While standardized initial treatment measures are essential, the treatment sequence must be tailored to each patient's unique circumstances.

Two cases stood out in our study. The first case involved a 16-year-old patient who had an exceptional outcome, surpassing expectations. She underwent AMT four months after the ocular burn and initially responded well to the isolated AMT, showing signs of satisfactory re-epithelization. However, during the follow-up, symblepharon formation and limbal stem cell failure were observed. Consequently, the patient underwent autologous limbal stem cell transplantation along with a new AMT, using our standardized technique. A symblepharon ring was also used for this patient. After a three-year follow-up, her VA improved from 1,30 logMAR (0.05 decimal) to 0.30 logMAR (0.5 decimal) (Figures 2 and 3).

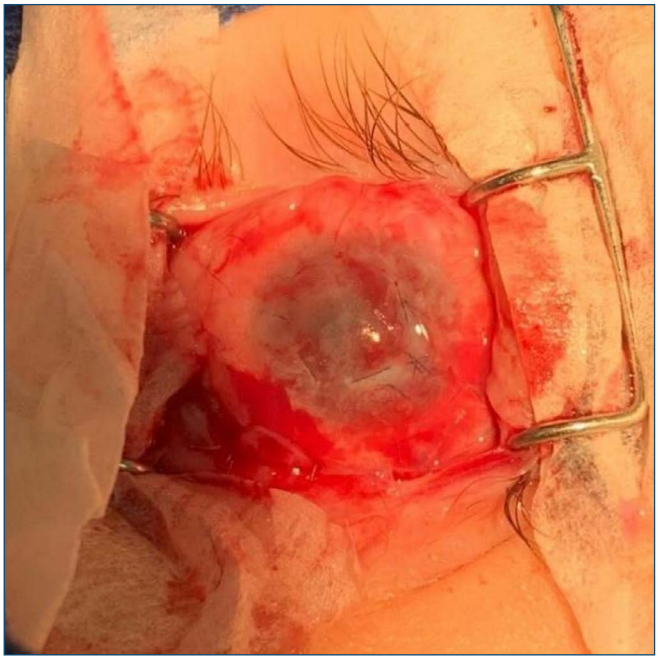


Figure 2. Amniotic membrane graft intraoperative.

The second case involved an 18-year-old patient who underwent isolated AMT nine days after the ocular burn. Her initial VA was 1.85 logMAR (0.014 decimal),

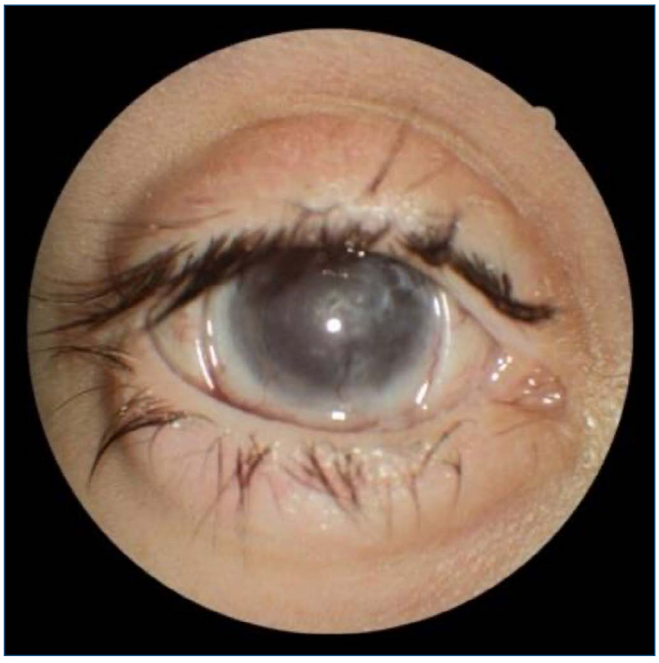


Figure 3. Amniotic membrane graft in-situ 30 days post-operatively.

which improved to 0.30 logMAR (0.5 decimal) at the 1-year follow-up, without requiring further procedures. Westekemper et al. showed that the timing of the AMT after trauma was significantly correlated to the final logMAR (Spearman's correlation p=0.006), with patients who received early intervention reaching a better final VA.[5]

These cases raise an important question for future studies: Does earlier intervention lead to the faster re-epithelialization, reduced risk of complications such as symblepharon, and increased chances of better VA? Unfortunately, a limitation of our study is that we do not have enough statistical data to confirm this hypothesis, which also reflects the delay in medical care within the public healthcare system.

CONCLUSION

Our study demonstrates the significant role of amniotic membrane transplantation as an adjunctive treatment in the management of ocular chemical burns. Despite the limitation of a small sample size, our findings indicate the benefit of amniotic membrane transplantation in improving the final visual outcome of patients. It is important to emphasize that in our country, where there is no access to synthetic membranes, the utilization of donated amniotic membranes plays a crucial role in the treatment of severe eye conditions, including chemical burns. Further research with larger cohorts is warranted to validate our results and explore the broader applicability of amniotic membrane transplantation in various ocular pathologies.

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