Efficacious Application of Amniotic Membrane in the Surgical Management of Combined Mine-Explosive Corneal Injuries

Aplicação Eficaz de Membrana Amniótica no Tratamento Cirúrgico de Lesões Corneanas por Explosão de Minas

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ABSTRACT

INTRODUCTION: Mine-explosive eye injuries, primarily affecting the cornea, occur due to several damaging factors from an explosive device. Several surgical methods stimulate corneal regeneration, including amnion transplantation, limbal stem cells from another healthy eye, induced pluripotent stem cells, mesenchymal stem cells, and bioprinting. Amniotic membrane transplantation prevents vascularization and fibrosis, improving reparative properties without causing transplant rejection, while the last three methods are still in clinical research stages.

Our objective was to evaluate the results of the surgical strategy for addressing combined mine-explosive corneal injuries.

METHODS: A retrospective analysis of 22 cases (22 eyes) of military personnel with mine-explosive corneal injuries (22 men, aged 30-45 years) revealed promising outcomes. All patients were submitted to a first surgical approach to stabilize the cornea, and then: first group: secondary surgical intervention including amniotic bio coating followed by conservative measures; versus second group: secondary surgical intervention followed by conservative measures and delayed amniotic membrane transplantation. Treatment effectiveness was assessed by analyzing indicators such as corneal syndrome (photophobia, lacrimation, blepharospasm), corneal edema (biomicroscopy/OCT), neovascularization, and transplant rejection over a 3-month period (July to October 2023).

RESULTS: During the three-month observation period, neither group exhibited signs of transplant rejection or corneal neovascularization. Group I had a mean time to improvement of 3.64 ± 0.77 days, with a median of 4 days. Group II had a mean time to improvement of 6.55 ± 1.30 days, with a median of 7 days. Both groups showed positive dynamics in reducing corneal edema over three months. Group I demonstrated significant reduction in corneal thickness both at 1 month and at 3 months (by 18.56%). Group II also showed a reduction in corneal thickness, but less pronounced (by 5.88%). These results indicate a significantly faster and more pronounced decrease in corneal edema in Group I compared to Group II over the three-month period ($t = 2.96$, $p = 0.008$).

CONCLUSION: Managing mine-explosive corneal injuries presents formidable challenges. The concurrent implementation of surgical intervention and amniotic bio-coating exhibited notable efficacy, mitigating corneal syndrome, and decreasing corneal edema and inflammation, thus promoting corneal regeneration.

KEYWORDS: Amnion/transplantation; Corneal Injuries/surgery; Explosions; Explosive Agents; Military Personnel; Ukraine.

RESUMO

INTRODUÇÃO: Lesões oculares causadas por minas explosivas afetam principalmente a córnea devido a vários fatores danosos. Métodos cirúrgicos estimulam a regeneração da córnea, incluindo transplante de âmnio, células-tronco limbares, células-tronco pluripotentes induzidas, células-tronco mesenquimais e impressão biológica. O transplante de membrana amniótica previne vascularização e fibrose, melhorando propriedades reparadoras sem causar rejeição, enquanto os três últimos métodos estão em fase de pesquisa clínica.

O nosso objetivo foi avaliar os resultados da estratégia cirúrgica para tratar lesões corneanas combinadas por minas explosivas.

MATERIAL E MÉTODOS: Uma análise retrospetiva de 22 casos (22 olhos) de militares com lesões corneanas causadas por minas explosivas (22 homens, com idades entre 30 e 45 anos). Os pacientes foram divididos em dois grupos: o primeiro recebeu tratamento cirúrgico secundário com biorevestimento amniótico e medidas conservadoras, enquanto o segundo foi submetido a cirurgia secundária com medidas conservadoras e transplante de membrana amniótica. A eficácia do tratamento foi avaliada analisando indicadores como síndrome da córnea (fotofobia, lacrimação, blefaroespasmo), edema corneano (biomicroscopia/OCT), neovascularização e síndrome de rejeição do transplante ao longo de um período de 3 meses (julho a outubro de 2023).

RESULTADOS: Durante três meses de observação, nenhum grupo apresentou sinais de rejeição ou neovascularização corneana. O Grupo I teve um tempo médio de melhoria de 3,64 ± 0,77 dias, com uma mediana de 4 dias. O Grupo II teve um tempo médio de melhoria de 6,55 ± 1,30 dias, com uma mediana de 7 dias. Ambos os grupos mostraram redução do edema corneano ao longo de três meses. O Grupo I teve uma redução significativa na espessura da córnea em 1 e 3 meses (18,56%). O Grupo II mostrou redução menos pronunciada (5,88%). Esses resultados indicam uma diminuição mais rápida e pronunciada no edema corneano no Grupo I comparado ao Grupo II ($t = 2,96$, $p = 0,008$).

CONCLUSÃO: A abordagem de lesões corneais por minas explosivas apresenta desafios consideráveis. A implementação simultânea de intervenção cirúrgica e bio-revestimento amniótico demonstrou uma eficácia notável, atenuando possíveis queixas corneanas, diminuindo o edema e a inflamação corneana, assim promovendo a regeneração da córnea.

PALAVRAS-CHAVE: Âmnio/transplante; Explosivos; Explosões; Lesões da Córnea/cirurgia; Militares; Ucrânia.

INTRODUCTION

Mine-explosive injuries arise from the concurrent multifactorial influence of the explosive device: encompassing the shock wave, the effects induced by fragments and particles of the explosive device, structural devastation, mechanical trauma, burns, and indirect injuries.[1-3]

The intricacies of corneal injuries in the context of armed conflicts, as opposed to peacetime scenarios, involve bilateral afflictions, a mine-explosive nature, concurrent damage to multiple ocular structures, the presence of intraocular foreign bodies, and combined injuries. These intricacies, stemming from the multifaceted impact of the causative weaponry, introduce challenges to the treatment and prognosis of such traumas.[4,5]

The number and severity of traumatic eye injuries during wars and local military conflicts continue to escalate.[5]

Optimizing the approach to patients with combined corneal injuries resulting from mine-explosive trauma and facilitating their subsequent rehabilitation and social ad-

aptation is crucial, given that corneal injuries are the most prevalent among visual organ damages.[6]

Explosive-induced penetrating corneal injuries necessitate prompt and expert primary surgical intervention. Given the gravity and multifaceted nature of the trauma, the implementation of a comprehensive set of subsequent therapeutic measures is paramount for enhancing reparative processes and facilitating rehabilitation.

There are several surgical methods aimed at stimulating corneal regeneration, including amnion transplantation, using limbal stem cells from a healthy eye, induced pluripotent stem cells, mesenchymal stem cells, and bioprinting. Limbal stem cell transplantation holds promise but is challenging to perform after mine-explosive injuries that affect each eye to different extents. This difficulty arises from the scarcity of healthy tissue in cases where both eyes lack sufficient limbal cells or have experienced severe injuries leading to anophthalmos. Induced pluripotent stem cells, mesenchymal stem cells, and bioprinting are currently undergoing clinical research and development stages.[2]

Among the surgical treatment strategies, amniotic membrane transplantation deserves special attention. The application of the amniotic membrane stands out for its ability to activate reparative regenerative processes, such as preventing epithelial apoptosis, enhancing the migration and differentiation of epithelial cells. Moreover, it contributes to the reduction of inflammatory processes, neovascularization, and corneal fibrosis.[7] This method is straightforward and practical, making it suitable for situations with limited resources and a large number of people involved. It helps efficiently calculate average arrival days and deviations, offering essential insights even in challenging circumstances.

Our objective was to evaluate the results of the surgical strategy for addressing combined mine-explosive corneal injuries.

MATERIAL AND METHODS

This study included 22 military personnel (22 eyes) with combined mine-explosive corneal injuries (22 men, aged 30-45 years). All patients received either early primary surgical treatment ((first surgical intervention 24 hours from being injured) or primary delayed surgical treatment (first surgical intervention more than 24 but less than 48 hours from being injured) at the previous evacuation stage. The mean time of admission to the department at the next evacuation stage (our hospital) was 4.41 ± 0.96 days.

Ophthalmological examination included visiometry, tonometry, biomicroscopy, ophthalmoscopy, conjunctival smear for flora and antibiotic sensitivity, anterior segment OCT, and photoregistration (when possible). All patients were divided into two groups according to the treatment strategy. Group I received "secondary surgical treatment" (repeated surgical intervention according to secondary indications - failure of previously applied sutures, insufficient adaptation of the wound edge) and therapeutic amniotic bio-coating of the cornea simultaneously, followed by subsequent conservative measures. Group II underwent a three-step approach, consisting of "secondary surgery", subsequent conservative measures, and amniotic membrane transplantation.

To assess the effectiveness and compare these strategies, the following indicators were examined: "corneal syndrome" (photophobia, lacrimation, blepharospasm), corneal edema (biomicroscopy/OCT - corneal thickness), neovascularization, transplant rejection syndrome, and statistical data analysis. The observation period was 3 months (from July to October 2023).

RESULTS

Within the spectrum of corneal injuries, penetrating wounds comprised 10 patients (45.45%), whereas non-penetrating injuries constituted 12 patients (54.54%). Regarding the presence of extraneous entities within the wounds, injuries featuring foreign bodies accounted for 68.18% - 15 patients (comprising 6 persons (40%) with deep-seated injuries and 9 persons (60%) with superficial injuries), while those devoid of foreign bodies constituted 7 patients (31.82%). In terms of localization, central injuries were noted in 4 patients (18.18%), paracentral in 8 patients (36.36%), central with a transition to the paraoptic zone in 7 patients (31.81%), and peripheral injuries in 3 patients (13.63%).

During the 3-month observation period, both groups exhibited no transplant rejection or corneal neovascularization.

In Group I, the average number of days until improvement (reduction in "corneal syndrome", corneal swelling, and inflammation) was 3.64 ± 0.77 days (SD), with a median of 4 days. In Group II, the average number of days until improvement was 6.55 ± 1.30 days (SD), with a median of 7 days.

Based on the statistical analysis of the corneal OCT data (Table 1), both groups showed a positive trend in the reduc-

Table 1. Dynamic of corneal thickness in both group.

Period	Group	Mean Corneal Thickness (μm) ± SD	95% Confidence Interval (μm)	Percentage Reduction	t-statistic	*p*-value
Before Treatment	Group 1	860.09 ± 92.59	(799.61, 920.57)	-	-0.23	0.824
Before Treatment	Group 2	869.72 ± 106.96	(804.77, 934.67)	-	-0.23	0.824
After 1 Month	Group 1	727.36 ± 120.04	(670.33, 784.39)	15.43%	-2.24	0.036 (p<0.05)
After 1 Month	Group 2	836.27 ± 95.26	(784.80, 887.74)	3.85%	-2.24	0.036 (p<0.05)
After 3 Months	Group 1	700.45 ± 84.80	(640.70, 760.21)	18.56%	-2.96	0.008 (p<0.05)
After 3 Months	Group 2	818.55 ± 93.57	(752.62, 884.47)	5.88%	-2.96	0.008 (p<0.05)

tion of corneal thickness, indicating a decrease in corneal edema. Before treatment, the mean corneal thickness was 860.09 µm (95% CI: 799.61 - 920.57) in Group I and 869.72 µm (95% CI: 804.77 - 934.67) in Group II (t = -0.23, p = 0.824), indicating no statistically significant difference between the groups.

After 1 month, there was an improvement in corneal thickness of 15.43% in Group I and 3.85% in Group II, with mean corneal thicknesses of 727.36 ± 120.04 µm and 836.27 ± 95.26 µm, respectively (t = 2.24, p = 0.036). This indicates a statistically significant difference between the groups.

After 3 months, Group I showed a further reduction in corneal thickness of 18.56%, while Group II exhibited a reduction of 5.88%. The mean corneal thicknesses were 700.45 ± 84.80 µm for Group I and 818.55 ± 93.57 µm for Group II (t = 2.96, p = 0.008). This demonstrates a statistically significant difference between the groups, with Group I experiencing a more pronounced decrease in corneal edema compared to Group II (Table 1).

CLINICAL CASE 1

Patient 1, transferred by the evacuation train to the Oleksandrivska Clinical Hospital of Kyiv in June 2023 with the diagnosis: "Mine-Explosive Trauma. Penetrating injuries of the cornea and sclera with intraocular foreign body. Burns of the conjunctiva, cornea of III-IV degree."

In the patient's medical history, approximately one week before, primary surgical intervention was carried out, subsequently transitioning to the next phase of evacuation.

Objective assessment: Visual acuity in both eyes was hand movement, with pronounced blepharospasm.

OD (oculus dexter): eyelids - edema, hyperemia, singed eyelashes, conjunctival injection; at 3 o'clock, an entry wound is observed. Cornea - lacerated wound extending from 3 to 9 o'clock in the horizontal meridian, slight edema; hazy anterior chamber, sluggish photoreaction, pink reflex, details unclear (Fig. 1).

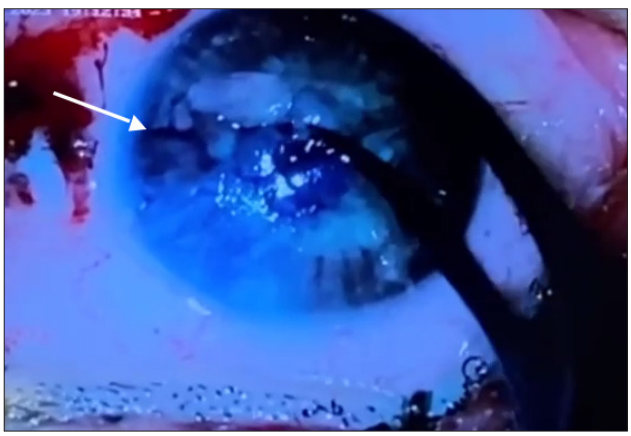

Figure 1. Explosive trauma. Penetrating injury to cornea. Conjunctival and corneal burns, III-IV degree.

* the arrow indicates corneal injury (from 3 to 9 hours); corneal eroded surface, severe swelling due to a burn.

"Secondary delayed surgical treatment" was performed for penetrating corneal injury, scleral revision, and therapeutic amniotic bio-coating of the cornea (Fig. 2). General and local anti-inflammatory and antibiotic therapy was prescribed.

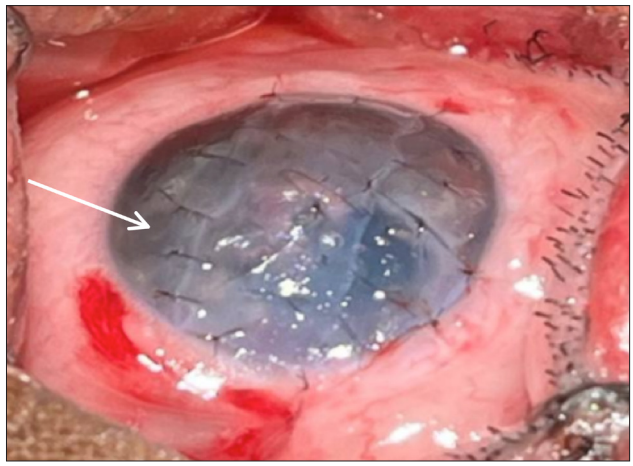

Figure 2. Status after "secondary surgical debridement" and amniotic membrane grafting: corneal and scleral wound adapted, amniotic membrane fixed.

* the arrow indicates the amnion fixed to the cornea.

Review after 7 days: Inflammatory manifestations are less pronounced. The amniotic membrane is fixed on the cornea, and the sutures are well-placed. "Corneal syndrome" is mild, with the anterior chamber of moderate depth and clear content. Photoreaction is sluggish, and the reflex is pink (Fig. 3).

Figure 3. Day 7 postoperative (objective assessment): the eye is calm, no "corneal syndrome" present.

* the patient calmly opens eye without lacrimation or blepharospasm.

Review after 3 months: The patient reports improvement, stating "can open eyes freely."

Objective findings: The eye is calmer, and the amniotic membrane is securely fixed. Optical coherence tomography (OCT) of the cornea shows that the amniotic membrane is well-apposed, and the sutures are fixed on the cornea. Scar formation is observed (Fig. 4).

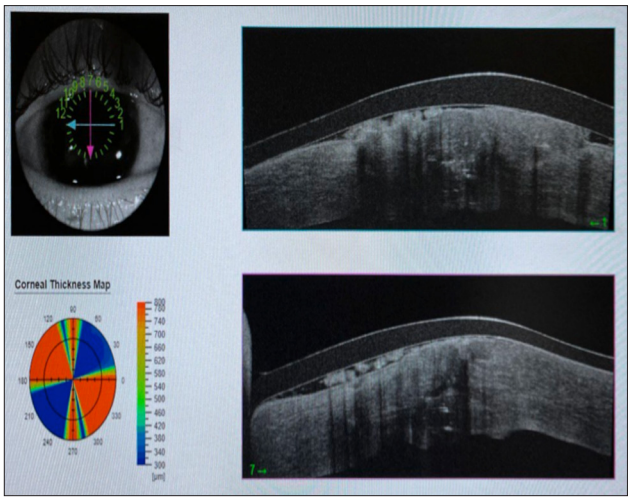

Figure 4. Three-month follow-up - OCT anterior segment. Amnion fixed. Formation of a dense scar.

There is positive dynamics in the regenerative processes of the cornea, and "corneal syndrome" is absent.

CLINICAL CASE 2

Patient 2, transferred for medical evacuation to the Main Military Medical Clinical Center of the State Border Guard Service of Ukraine in July 2023, was diagnosed with: Mine-explosive trauma. Penetrating corneal injury with prolapse of intraocular membranes with gunshot fragment foreign bodies, hyphema, traumatic cataract, and hemophthalm in the right eye.

At the previous stage of evacuation, the following procedures were performed: primary surgical treatment of corneal injury with foreign body removal; posterior closed vitrectomy with silicone endotamponade, and a reoperation due to recurrent retinal detachment.

Objective findings:

VISUS OD = 0.01 not corrected

VISUS OS = 1.0 not corrected

OD: Pronounced blepharospasm, conjunctival injection, corneal edema, erosion in the area of corneal sutures, nodal sutures are ineffective, deep anterior chamber, clear content, mydriasis, aphakia, vitreous body – avitria, silicone tamponade.

According to the anterior eye segment optical coherence tomography (AS-OCT), the scar formation was insufficient. "Secondary surgical treatment" of penetrating corneal injury was performed, followed by conservative therapy (Fig. 5).

Follow-up after 7 days: The eye is irritated, with pronounced blepharospasm, 'unable to open eyes,' tearing, significant conjunctival injection, corneal swelling, well-placed sutures on the cornea, deep anterior chamber, clear content, mydriasis, aphakia, vitreous body avitria, and silicone tamponade.

Considering the severity of the condition and the absence of positive dynamics, therapeutic amniotic bio-coating of the cornea was performed.

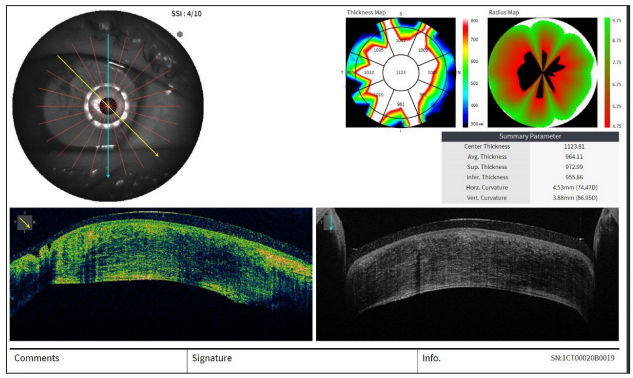

Figure 5. Status after "secondary surgical treatment", next stage: amniotic membrane grafting (OCT anterior segment - scar formation, corneal edema).

Observation after 7 days revealed patient subjective improvement, experiencing easier eye opening, and reduced tearing. Continued conservative therapy and monitoring were in progress.

Review after 3 months indicated a calm eye, normal pupil size, pale pink conjunctiva, well-fixed amnionitic membrane on the cornea, deep anterior chamber with clear content, mydriasis, aphakia, vitreous body avitria and silicone tamponade (Fig. 6).*

* In this article, two of the most challenging cases of combined mine-explosive corneal injuries were presented to demonstrate practical experience with amnion transplantation in military personnel.

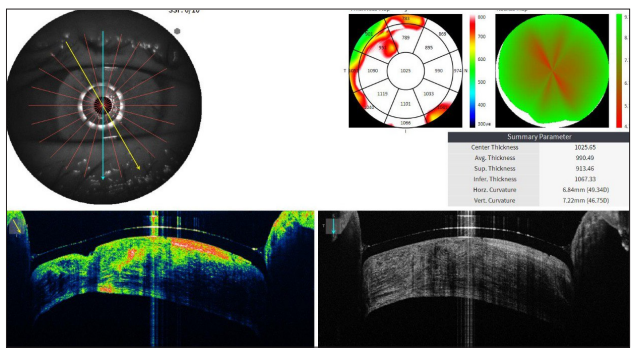

Figure 6. Three-month follow-up - OCT anterior segment- reduction of corneal edema, formation of a dense scar.

DISCUSSION

The amniotic membrane, consisting of amniotic epithelium, basement membrane, and stroma, is rich in metabolically active components, including lipids, polysaccharides, and growth factors. These elements contribute to its therapeutic effectiveness in promoting tissue epithelialization and regeneration. The diverse biological properties of the amniotic membrane make it a valuable tool in addressing ocular trauma and inflammation, showcasing its potential in fostering healing and regeneration.

The amniotic membrane exerts a multifaceted impact on the regenerating corneal epithelium, facilitating the

migration of epithelial cells, enhancing adhesion of basal epithelial cells, and playing a crucial role in stimulating cell differentiation while inhibiting apoptosis.[7]

According to clinical randomized trials, the effectiveness of amniotic membrane transplantation in cases of corneal injuries of moderate severity due to thermal or chemical causes is highest within the first 7 days. A significant reduction in `corneal syndrome` and inflammatory activity is observed during the initial week post-transplantation. In the short term, after 3 months, the efficacy of medical therapy compared to the action of the amniotic membrane, combined with conservative treatment, is found to be similar based on biomicroscopy, and "corneal syndrome" indicators(epiphora, blepharospasm, photophobia). However, in cases of severe injuries, the effectiveness of amniotic membrane application is inconclusive, potentially attributed to the severity of the overall ocular condition and the challenge of assessing the amniotic membrane's impact on the healing process.[7]

The early transplantation of the amniotic membrane in various corneal injuries facilitates the acceleration of healing processes, addressing both superficial epithelial defects and deeper stromal injuries of diverse etiologies. It serves to reduce the intensity of the inflammatory response and mitigates the necessity for further surgical interventions, including keratoplasty or corneal transplantation.[3,8]

Alternatively, transplantation of the amniotic membrane carries the risk of infection, similar to any other eye surface surgery. Additional concerns involve potential implant displacement, complete absorption of the membrane, necessitating subsequent surgeries.[9]

Based on the conducted study, were compared two approach to treatment: Group I – simultaneously surgery and amnion bio-coating, Group II – surgery, conservative measures and then amnion bio-coating. After conducting a statistical analysis of the obtained, it was established significant reduction in `corneal syndrome` and inflammatory processes is observed within 3.64 ± 0.77 days in Group I and 6.55 ± 1.30 days in Group II. This may be associated with the delayed therapeutic amniotic bio-coating and the severity of the mine-explosive trauma.

The mean corneal thickness indicators before treatment were similar in both groups (860.09 μm in the first group and 869.72 μm in the second group), indicating no statistically significant differences between the groups before the start of treatment ($t = -0.23$, $p = 0.824$). After 1 month of treatment: in Group I, the mean corneal thickness decreased by 15.43% (727.36 μm), while in Group II - decreased by 3.85% (836.27 μm). The difference between the groups was statistically significant ($t = 2.24$, $p = 0.036$), indicating the advantage of the simultaneous treatment approach. After 3 months of treatment: in the Group I, the corneal thickness decreased by 18.56% (700.45 μm), while in Group II - decreased by 5.88% (818.55 μm). The difference between the groups remained statistically significant ($t = 2.96$, $p = 0.008$), confirming the long-term effectiveness of the simultaneous approach.

The obtained results demonstrate a significant advantage of simultaneous surgical intervention and corneal bio-coating compared to the sequential approach. Group I experienced faster and more substantial reductions in corneal edema and "corneal syndrome". This can be attributed to faster corneal regeneration and reduced inflammatory response, leading to quicker recovery.

Despite the positive outcomes, this study has limitations including a small sample size of 22 patients and a short three-month observation period, which may affect the overall statistical power and long-term assessment of treatment effectiveness.

The study data confirm the high effectiveness of simultaneous surgical intervention and corneal bio-coating in treating mine-explosive injuries. This approach has statistically significant advantages over the sequential approach in both the short and long term. However, further studies with larger sample sizes and longer observation periods are necessary to confirm these findings and develop new treatment protocols.

It should be noted that while increasing the sample size is possible, the observation period cannot be extended at a single medical facility since military personnel must receive high-quality, comprehensive care in the shortest possible time before being referred to the next stage of evacuation for further treatment and rehabilitation. Therefore, this study can serve as a basis for future research with larger sample sizes, necessary to confirm the results and improve treatment strategies in armed conflict situations with large numbers of casualties and limited resources.

CONCLUSION

Corneal injuries of mine-explosive nature pose challenges for treatment and subsequent rehabilitation. The crucial aspect is the provision of comprehensive, high-quality surgical assistance at the earliest opportunity. It is noteworthy that the presented technique involves not only adapting the edges of the corneal wound with the removal of foreign bodies but also applying therapeutic amniotic bio-coating of the cornea.

The early transplantation of the amniotic membrane proves to be effective in addressing "corneal syndrome" issues and reducing inflammatory phenomena. The absence of vascularization and fibrosis allows for planning future reconstructive interventions in the deep layers of the eye, contributing to achieving better functional outcomes and minimizing vision-related disability.

Among the benefits of using the amniotic membrane, it is pertinent to highlight the potential for subsequent phototherapeutic laser keratectomy aimed at enhancing corneal transparency and optimizing visual outcomes. It is imperative to underscore that this technique is applicable solely to the anterior corneal segments characterized by stromal opacities, contingent upon the preservation of at least one-third of its thickness (with a minimum thickness of 250 μm).[10]

CONTRIBUTORSHIP STATEMENT / DECLARAÇÃO DE CONTRIBUIÇÃO

AC, TP, YO, NB e MM: Responsible for gathering the data. AC, NB: Responsible for creating the manuscript.

AC, TP, NB: Supervised this project and contributed with their expertise to its conclusion.

All authors read and approved the final manuscript.

AC, TP, YO and MM: Responsável pela recolha dos dados.

AC, NB: Responsáveis pela criação do manuscrito.

AC, TP, NB: Supervisionaram este projeto e contribuíram com os seus conhecimentos para a sua conclusão.

Todos os autores leram e aprovaram o manuscrito final.

RESPONSABILIDADES ÉTICAS

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.

Fontes de Financiamento: Não existiram fontes externas de financiamento para a realização deste artigo.

Confidencialidade dos Dados: Os autores declaram ter seguido os protocolos da sua instituição acerca da publicação dos dados de doentes.

Proteção de Pessoas e Animais: Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pela Comissão de Ética respon-sável e de acordo com a Declaração de Helsínquia revista em 2013 e da Associação Médica Mundial.

ETHICAL DISCLOSURES

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2013).

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