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Original Article

Graft Survival after Lamellar Keratoplasty without Limbal Stem Cell Transplantation in Patients Exposed to Chemical Warfare

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Abstract

Background: Keratopathy is a common complication following exposure to chemical warfare, namely mustard gas. In recent years, a quest was undertaken to seek an effective and uncomplicated corneal transplantation method by ophthalmologists nationwide. In our study, we report our results following lamellar keratoplasty (LK) without limbal stem cell transplantation in patients with delayed-onset mustard gas keratopathy (DMGK).

Methods: Thirty-one eyes of 22 veterans with DMGK who underwent conventional LK were assessed. The presence of limbal stem cell deficiency (LSCD) was proven clinically as well as by impression cytology. The results were evaluated based on best spectacle-corrected visual acuity (BSCVA), refractive error (RE), corneal clarity, and corneal graft survival rate.

Results: The mean age of patients at the surgery was 52.93±5.8 years, and the mean follow-up was 36.25±20.65 months. The mean preoperative BSCVA was 1.67±0.32 logarithm of the minimal angle of resolution unit (Log MAR), significantly improved, reaching 0.32±0.22 Log MAR after surgery (p = 0.001). The mean preoperative spherical equivalent (SE) was 2.47±1.17 diopters, significantly increasing to 3.5±1.08 diopters after suture removal was complete (p = 0.001). Epithelial graft rejection occurred in 9.67% of eyes (3/31) and was treated successfully with topical eye drops. At the end of the follow-up period, no signs of graft rejection were found, but three eyes had mild central corneal opacity.

Conclusion: According to the results of our study, LK without LSC transplantation can be successful in patients with DMGK because of partial rather than complete LSCD.

Keywords: Sulfur Mustard, Lamellar Keratoplasty; Limbal Stem Cell, Delayed-Onset Mustard Gas Keratopathy.

Introduction

Sulfur mustard (SM) and Nitrogen mustards are two forms of mustard gas that can damage the hemopoietic, gastrointestinal, and central nervous systems. These are exposed organs such as skin, respiratory tracts, and eyes [1]. Nitrogen mustard is commonly used for the chemotherapy of neoplasms and is rarely used in chemical warfare. In contrast, sulfur mustard, which can last longer, was used as a weapon of mass destruction in World War I in 1917 [2, 3]. The most recent use of this agent was in the Iraq-Iran war, which was used against Iranian forces, and about 100,000 people sustained chemical injuries [4, 5]. The eyes are the most susceptible tissue to SM, and depending on the extent of exposure, different parts of the eye, including the eyelids, conjunctiva, and especially the cornea, are damaged. Ocular complications (pain, tearing, photophobia, and severe blepharospasm) usually improve after a few weeks. However, in some patients, the eye manifestations do not resolve and progress to a chronic phase. Late corneal involvements may occur one to 30 years after initial contact with SM and are known as delayed-onset mustard gas keratopathy (DMGK) [6, 7]. This keratopathy causes a wide range of ocular complications from mild to severe, including dry eye, chronic photophobia, decreased vision,

conjunctival vessel changes, limbal ischemia, limbal stem cell deficiency (LSCD), opacity, corneal thinning, scarring, neovascularization, lipid and amyloid deposition, and corneal melting [7-12]. The pathogenesis of chronic and delayed MGK still needs to be fully discovered. However, it may be due to severe degeneration of the basement membrane zone, persistent corneal edema, and stromal abnormalities [13-15]. Various medical and surgical interventions treat dry eye, limbal ischemia, LSCD, and corneal involvements in affected individuals. This medication is not limited to prescribing artificial tears, topical or systemic blepharitis treatment, punctual occlusion, tarsorrhaphy, amniotic membrane transplantation, keratolimbal allograft, and keratoplasty. Penetrating keratoplasty (PK) or lamellar keratoplasty (LK) is essential when there is severe visual impairment and extended corneal involvement. Due to the high risk of graft failure after PK due to inflammation and vascularization of the cornea, conventional LK is recommended in cases of central corneal opacity and the presence of normal endothelial cells. Nevertheless, in some cases where there is excessive corneal thinning and complete corneal involvement, PK is the best choice [8, 16-21].

Two previous studies showed that since there are degrees of LSCD in the eyes with DMGK, LSC transplantation can improve the results of LK, and the simultaneous procedure is preferred to sequential surgery [22, 23].

It should be noted that in most patients suffering from DMGK, LSCD is partial [24]. So LSC transplantation may not be essential, and it is wise to avoid some adverse effects in the recipient's eyes, including epithelial erosion, intraocular pressure elevation, and immunosuppressant side effects [25].

This study aimed to evaluate corneal transplantation results in patients who suffered from DMGK without stem cell transplantation and report the outcomes during a three-year follow-up.

Methods

In this no comparative, interventional study, 31 eyes of 22 sulfur mustard-injured patients in the Iran-Iraq war (between 1980 and 1988) were evaluated at the vision health research center in Tehran, Iran.

Since Iran is one of the biggest victims of chemical warfare and there are many chemical casualties in Iran, the selection of cases is based on the experience of the authors who have studied these rare patients for years after the Iran-Iraq war.

According to an expert ophthalmic surgeon (K.J), all of our participants had DMGK, and the corneal involvement was so severe that they needed LK. Indications for LK were decreased visual acuity, irritation, and photophobia caused by corneal opacity, severe corneal thinning, abnormal deposits, or a combination of these with a normally functioning endothelium.

Partial stem cell deficiency in all patients by observing the epithelial defect, stromal inflammation, corneal neovascularization, conjunctival epithelial growth, and corneal opacification [Figure 1], as well as in 10 randomly selected eyes using impression cytological analysis [Figure 2] was detected.

A complete history, including sex, age, time of exposure to mustard gas, ocular symptoms, ophthalmic and systemic medications, systemic diseases, and history of any ocular or systemic surgery, was taken from all patients.

Patients with corneal endothelial involvement, total LSCD, and high IOP were excluded from this study.

Preoperative refractive error (RE) and best spectacle-corrected visual acuity (BSCVA) were measured in all patients. Patients underwent slit-lamp bio microscopic examination to assess the corneal status, tear meniscus height, and intra-ocular pressure (IOP). Finally, dilated fundoscopy was performed for all patients.

All patients underwent LK surgery without stem cell transplantation, and all surgeries were performed by the same surgeon (K.J). Patients were followed up post-operatively every week for the first month, then monthly for up to one year, and every three months after that. The RE, BSCVA, and IOP values were measured in each follow-up visit, slit-lamp bio-microscopic examinations were repeated, and graft clarity was evaluated.

Slit lamp photographs were also taken before and after the operation.

Surgical intervention, lamellar keratoplasty

First, the patients underwent general anesthesia. The cornea was thoroughly washed with the saline solution, and the size of the area to be removed was determined. A Barron Hesburgh suction trephine was used to trephine the recipient's cornea, and the cornea was scraped layer-by-layer by the crescent blade to reach the deep stroma. To create a soft, clear recipient bed, the removal process continued until the cornea was completely clear and no scar or deposit was observed. The donor cornea, approximately 0.5 mm more significant than the recipient's diameter, was cut with a Hesburgh Barron punch, then the corneal endothelium was removed and placed on the recipient's eye. It is then sutured to the recipient bed using a 10.0 nylon suture. Our suturing method was mostly single-running sutures and, in some cases, interrupted sutures.

After transplant surgery, the patient’s punctum was cauterized after transplant surgery if it had not been closed. Moreover, lateral tarsorrhaphy was performed in all patients. The patient was examined the next day, and the appropriate medications were prescribed.

Post-operation phase. After surgery for all patients, betamethasone 0.1% (Sina Darou, Tehran, Iran) topical eye drops every 6 hours for two weeks and then tapered over six months. Ciprofloxacin 0.3% (Sina Darou, Tehran, Iran) every 4 hours for a week were prescribed. Also, preservative-free lubricant was administered every 2 hours for a week to a year, depending on the patient’s condition. Sutures were not usually removed until one year after surgery. After one year, we removed the sutures selectively based on the refractive error and the amount of corneal stigmatization.

Ethical consideration. The Ethics Committee of Baqiyatallah University of Medical Sciences (IR. BMSU.1399.060) approved the study protocol, which was conducted following the tenets of the Helsinki Declaration. All participants signed a written informed consent before the treatment.

Statistical analysis

Statistical analysis was carried out using SPSS software version 20 (SPSS for windows, Chicago, IL). Mean ± standard deviation (SD) was used to present descriptive data. The data were compared with normal distribution using the Kolmogorov-Smirnov goodness of fit (K-S test). To evaluate the normally distributed data, the paired-samples t-test was conducted; otherwise, the equivalent nonparametric test (Wilcoxon Signed Ranks Test). P-values less than 0.05 ($P < 0.05$) were considered significant levels.

Result

5) were considered significant levels.

Results

Our study included 31 eyes of 22 patients (all male) with DMGK who were exposed to mustard gas more than 30 years before the intervention. At the time of the LK surgery, the mean age of these cases was 52.93 ± 5.8 years (range 38-62 years), and the mean duration of postoperative follow-up was 36.25 ± 20.65 months (range, 12-72 months).

Preoperative slit lamp examinations showed chronic blepharitis and varying degrees of dry eye in all patients. IOP was normal in all patients. In corneal evaluation, peripheral and paracentral thinning of corneal stoma in 38.7% (12/31) of eyes was observed. Also, corneal opacity, neovascularization, and lipid or amyloid deposition were seen in 32.2% (10/31), 32.2% (10/31), and 29% (9/31) of cases, respectively.

The average BSCVA before surgery was 1.67 ± 0.32 logarithm of the minimum angle of resolution (log MAR) units, ranging from 2.09 to 1.22, which significantly improved and reached 0.32 ± 0.22, ranging from 1 to 0.8, three months after surgery ($p = 0.001$). The mean spherical equivalent refractive error before the operation was 2.47 ± 1.17 D, significantly increasing to 3.5 ± 1.08 D after the operation ($p = 0.001$). The refractive error was measured at least three months after the surgery (Table 1).

Table 1: Statistical analysis to test the mean difference between before and after measurements of BSCVA and SE

Variable	N	Mean	SD	Min	Max	P-Value
Pre-op BSCVA	31	1.67	0.32	1.22	2.09	0.001
Post-op BSCVA	31	0.32	0.22	0.10	1.00	
Pre-op SE	31	2.47	1.17	0.00	4.50	0.001
Post-op SE	31	3.50	1.08	1.50	6.00	0.001

Cytological impression was performed in 32% (10/31) of the eyes with clinical signs of LSCD, and the

analysis showed the presence of goblet cells in at least a quarter of the cornea [Fig2].

We did not find any severe squamous metaplasia or conjunctival intraepithelial neoplasia. Preoperative symptoms were decreased vision, photophobia, dry eye, and foreign body sensation. After surgery, 77.4% (24/31) of these patients are satisfied due to reduced symptoms. Epithelial graft rejection was observed in 9.67% (3/31) of eyes after keratoplasty, which was successfully treated with betamethasone 0.1% topical eye drop (Sina Darou, Tehran, Iran).

Interface vascularization was observed in 6.4% (2/31) of eyes, and peripheral scar formation was seen in 9.67% (3/31) of eyes. None of the patients demonstrated evidence of graft rejection. However, 9.67% (3/31) of eyes have mild central corneal opacity in postoperative examinations. The postoperative complications are summarized in Table 2. The following figures show some of the eyes that underwent LK surgery [Figure3; A, B, C, and D].

Due to the particular circumstances of these patients and their constant need for medical care, all patients were present during the follow-up period, and none of them were missed.

Table 2: Complications after lamellar Keratoplasty without limbal stem cell transplantation in chemically injured patients.

Post-operative findings	Percent (number/total eyes)
peripheral and paracentral thinning of corneal stoma	38.7% (12/31)
corneal opacity	32.2% (10/31)
neovascularization	32.2% (10/31)
lipid or amyloid deposition	29% (9/31)
Epithelial graft rejection	9.67% (3/31)
Interface vascularization	6.4% (2/31)
peripheral scar formation	9.67% (3/31)
mild central corneal opacity	9.67% (3/31)
graft rejection	0%

Figure 1: Image showing the limbal stem cell deficiency caused by mustard gas. The normal epithelium is replaced by conjunctival epithelium, which lead to corneal neovascularization and opacification.

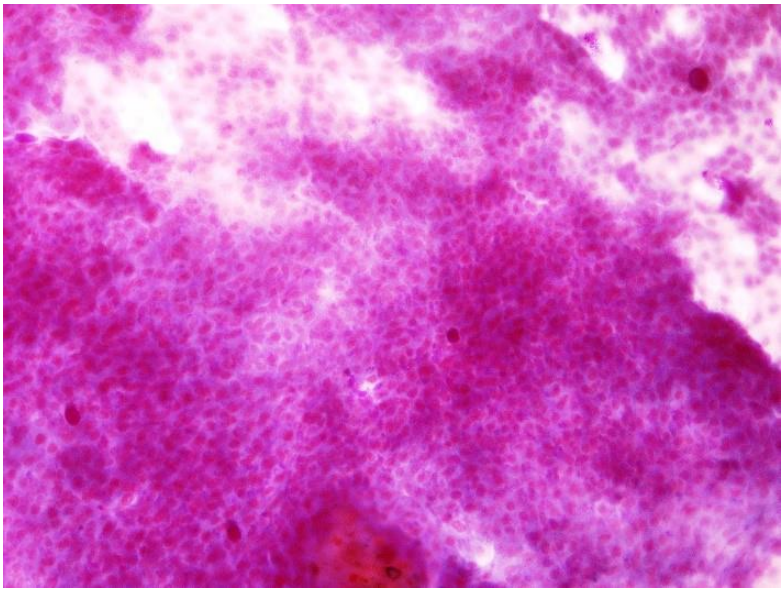

Figure 2: Results of corneal impression cytology in a patient with delayed-onset mustard gas keratopathy showing corneal epithelial cells with marked conjunctivalization and the presence of some goblet cells, modified pas staining ×100.

Figure 3: Images showing graft clarity approximately 3 years after lamellar keratoplasty for mustard gas keratitis in patients with partial limbal stem cell deficiency.

Discussion

To date, 250 cases of DMGK have been found in approximately 100000 SM affected veterans [26, 27]. Their preferred method for treating patients with these rare conditions depends on the type and severity of the ocular involvements. It includes a variety of interventions to improve symptoms, such as chronic blepharitis, dry eye, stem cell deficiency, and corneal complications [8, 28]. Corneal transplantation is indicated in severe corneal thinning, opacity, and reduced vision [21]. Formerly, PK

was performed on these individuals. In a study by Javadi et al. [16], graft clarity after PK was reported to be 77.3% in patients who suffered from MGK, with about 50% of eyes having endothelial or sub epithelial rejection episodes. In the other study, Feizi et al. [18] compared the results of PK and LK surgery in mustard gas-induced eyes. They reported that in 27 eyes that underwent PK, the graft survival rate was 77.3%, while in 51 eyes with LK, it was 91.7%; during the mean follow-up of 67.5 and 76.1 months, respectively.

Transplant rejection is a high risk due to chronic inflammation and vascularization of the cornea after PK in the eye with MGK [21].

Based on these results and clinical and histological findings, which show that most eyes with MGK have anterior corneal involvement and the corneal endothelium remains undamaged, conventional LK can be an appropriate method in these affected eyes [17]. On the other hand, our experience has indicated that deep anterior lamellar keratoplasty is difficult in these patients using the extensive bubble technique because it is difficult to separate the Descemet's membrane from the stroma. It may be due to alterations in the corneal stroma due to corneal inflammation, corneal scarring, and lipid and amyloid deposits.

We now know chemical injuries to mustard gas lead to varying degrees of LSCD, and this complication is one of the causes of DMGK [29, 30]. Stem cell defects result in conjunctivalization of the cornea with vascularization, the appearance of goblet cells, and the formation of an irregular and unstable epithelium stem cell transplantation, which irritates the eye and reduce vision. Therefore, stem cell transplantation is required to treat these complications [31]. Until now, studies have shown that stem cell transplantation effectively improves the results of LK surgery in patients with DMGK, and the results of the simultaneous approach are better than staged interventions [22, 23].

Impression cytology findings and clinical examination have changed our views of LSC transplantation. We found that LSCD is partial in chemical injuries and is more common in the area of the eyelid fissure (in the nasal and temporal region) that is most exposed. These findings are confirmed in a study by Baradaran-Rafii and colleagues using impression cytology. They stated that in DMGK, the severity of corneal clinical manifestations is higher in the nasal and temporal zones, and LSCD is initially localized than the total in chemical veterans [24].

The outcomes of this study indicated that lamellar keratoplasty without LSC transplantation could be the preferred method in MGK patients with partial LSCD. We have not observed any evidence of graft rejection or failure during the three-year follow-up period. Also, visual acuity and the patient's symptoms were improved. Furthermore, there was no evidence of exacerbations in postoperative examinations, and no new signs of mustard gas activity were found in our study population. The only observed complication was epithelial graft rejection in three eyes which was treated with topical betamethasone 0.1% eye drops.

One of the advantages of this method was the prevention of long-term use of systemic immunosuppressant after stem cell transplantation which can exacerbate systemic problems of chemical warfare, such as respiratory injuries [32]. After stem cell transplantation and prolonged systemic immunosuppression, we have experienced patients with developed lung infections and cytomegalovirus in two cases (data not shown).

Our study had several limitations. For instance, we did not perform impression cytology analysis in all subjects, after LK, and in postoperative periodic examinations. The status of stem cells was assessed only by bio-microscopic examination and photo-slit images. Long-term follow-up is required for these rare patients. In all of these patients, our examinations and evaluations will continue, as many questions about this rare DMGK complication remain, and more research is needed on its exact nature and pathophysiology. We will regularly review graft clarity and stem cell status in these patients and hope to report 5-year and 10-year results in future studies.

Conclusion

According to our results, conventional lamellar keratoplasty alone is an acceptable surgical method for corneal and limbal involvement in delayed-onset mustard gas keratopathy due to partial LSCD.

Abbreviations

Lk: lamellar keratoplasty
DMGK: delayed-onset mustard gas keratopathy
BSCVA: best spherical corrected visual acuity
RE: refractive error
Log MAR: logarithm of the minimal angle of

resolution
PK: penetrating keratoplasty
IOP: intra ocular pressure
D: diopter

Authors' contributions

Designed research and wrote the manuscript: SHB, SHD. Collected and analyzed data.: HA. Designed research and performed study: KHJ. The authors read and approved the final manuscript.

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Disclosure statement

The authors declare that they have no competing interests.

Ethical Statement

The Ethics Committee of Baqiyatallah University of Medical Sciences (IR. BMSU.1399.060) approved the study protocol, which was conducted in accordance with the tenets of the Helsinki Declaration. All participants signed a written informed consent before the treatment.

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