

# Limbal Stem Cell Deficiency in Chronic and Delayed-onset Mustard Gas Keratopathy

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**Purpose:** To evaluate limbal stem cell deficiency (LSCD) using impression cytology in patients with chronic and delayed-onset mustard gas keratopathy (MGK).

**Design:** Prospective observational case series.

**Participants:** Thirty-five eyes of 18 patients (all male) with MGK were included.

**Methods:** A consecutive series of patients with MGK underwent impression cytology. Finding of goblet cells on the corneal side of specimens was considered as LSCD. Severity of corneal clinical manifestation was graded as mild, moderate, and severe in each quadrant. Relation between impression cytology findings and clinical grading was evaluated.

**Main Outcome Measures:** Impression cytology findings and clinical grading.

**Results:** There was LSCD in at least 1 quadrant of cornea in all 35 eyes (100% of cases). No differences were found between impression cytology findings (positive vs. negative for corneal goblet cells) among different quadrants ( $P = 0.378$ ). Clinical grading was the same between nasal and temporal quadrants ( $P = 0.266$ ) and between superior and inferior quadrants ( $P = 0.263$ ). By combining superior and inferior quadrants (vertical zone) and nasal and temporal quadrants (horizontal zone), corneal clinical grading was more severe in horizontal versus vertical zones ( $P < 0.001$ ). There was no relation between LSCD and corneal clinical severity ( $P = 0.893$ ).

**Conclusions:** A varying degree of LSCD was demonstrated in all patients with chronic or delayed-onset MGK using impression cytology. Corneal clinical manifestations are more severe in nasal and temporal quadrants. There was no relation between impression cytology findings (positive vs. negative for goblet cells) and corneal clinical grading. Other factors, such as perilimbal conjunctival ischemia, may play a role.

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Preservation of integrity of corneal epithelium is dependent on the cells with self-renewal properties. Stem cells located at the limbus are the ultimate source for regeneration of the corneal epithelium in the normal and traumatized states.<sup>1–4</sup> The pathologic state of limbal stem cell deficiency (LSCD) can be caused by the destructive loss of limbal epithelial stem cells or the dysfunctional limbal stroma (niche).<sup>5–7</sup> Signs of LSCD include corneal conjunctivalization, chronic ocular surface inflammation, and neovascularization. Epithelial defects are common in the conjunctivalized corneal surface and may lead to corneal ulceration, scarring, and loss of vision.<sup>8,9</sup>

Impression cytology is a useful noninvasive technique, in which the first layer or the 2 outermost layers of the ocular surface epithelium are removed and studied to determine the state of the conjunctival surface. It is the gold standard for confirmation of the conjunctivalization of the cornea. Finding goblet cells on the surface of the cornea is considered as a definitive diagnosis of corneal conjunctivalization and LSCD.<sup>10</sup> Impression cytology has also been used to classify the severity of squamous metaplasia.<sup>11</sup>

Mustard gas was extensively used by Iraqi forces against Iranian troops during the Iraq–Iran war (1980–1988), and more than 100 000 individuals were exposed to it.<sup>12–14</sup> Approximately 0.5% of exposed patients will develop late ocular complications that are usually progressive and per-

manent, leading to reduction of visual acuity and even corneal blindness. Late manifestations may appear after a variable latent period (delayed-onset form) or follow immediate lesions in the form of persistent smoldering inflammation (chronic type). In delayed and chronic forms, main features are categorized to conjunctival, limbal, and corneal involvement.<sup>13,15</sup> Mustard gas-induced keratopathy, which manifests with a combination of impaired corneal sensation, recurrent/persistent epithelial erosions, damaged limbal vasculature, and neovascularization, produces corneal irregularity and thinning that may extend to deep layers of the cornea, producing descemetocoeles and occasionally perforation.<sup>13,16–18</sup>

Although there are some reports suggesting progressive LSCD as a contributing factor to corneal manifestations associated with mustard gas,<sup>13,19,20</sup> to the best of our knowledge, no study has described the actual pathogenesis of corneal manifestations in patients with mustard gas keratopathy (MGK). This is the first report of LSCD using impression cytology in patients with chronic or delayed-onset MGK.

## Materials and Methods

This study was performed on a consecutive series of mustard gas chemically injured patients with chronic or delayed keratopathy. The study was conducted at Labbafinejad Medical Center, Tehran,

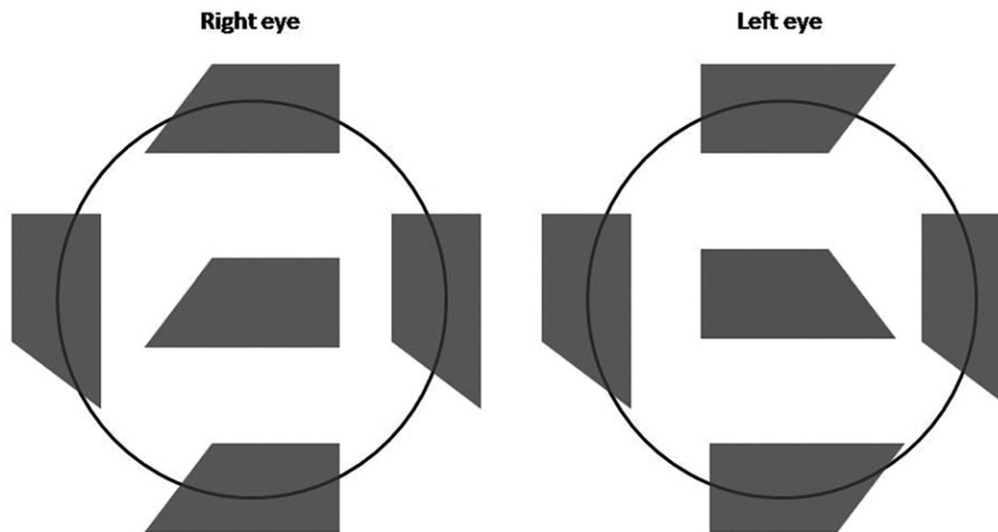


Figure 1. Orientation of cut papers for impression cytology.

Iran, from 2006 to 2008. The study protocol was based on the tenets of the Declaration of Helsinki and approved by the institutional review board and ethics committee of the Ophthalmic Research Center. Mustard gas exposure was confirmed by reviewing documented medical records and characteristic clinical manifestations of the patients. Patients with previous ocular surgeries were excluded. Informed consent was obtained from all patients.

All patients had complete eye examinations, including visual acuity measurement with or without spectacle correction, biomicroscopic examination, intraocular pressure measurement, and funduscopy. All patients had digital photography (Imagenet, Topcon SL-8Z, Tokyo, Japan) from both eyes. Impression cytology was performed in all cases by one of the authors (ABR).

All digital photographs were reviewed by 2 of the authors (ABR and MAJ), and their clinical grades were categorized. Corneal quadrant involvement was graded as mild, moderate, or severe. Conjunctival vessel changes, including telangiectasia, tortuosity, and segmentation, were characteristics of the mild group. The adjacent corneal quadrant was clear in this group. Limbal ischemia and peripheral vessel invasion with or without corneal opacity were features of the moderate group. If previous findings were accompanied by corneal thinning and melting, it was considered as severe. The relation between corneal clinical grading and impression cytology findings (positive vs. negative for goblet cells) was evaluated.

Data were analyzed using SPSS statistical software (version 15, SPSS Inc., Chicago, IL). Logistic regression and ordinal logistic regression were used for relation between and evaluation of the impression cytology findings and corneal clinical grading, respectively. Marginal regression (based on the generalized estimating equation approach) was used to consider the correlation among quadrants in these aforementioned regressions.

## Impression Cytology

After instilling an anesthetic drop (tetracaine 0.5%) into the conjunctival sac of the affected eye, excess moisture was cleared. Trapezoid, end-pointed, small strip papers were cut using a cellulose acetate filter (47 mm, pore size 0.45  $\mu\text{m}$ ) (Schleicher & Schuell Microscience GMBH, Dassel, Germany). By using the standard method, four 5 $\times$ 5-mm cut papers were applied on the superior, inferior, nasal, and temporal quadrants of the limbus for

a few seconds according to Tseng's<sup>21</sup> modified method. Another paper was used for sampling the center of the cornea. Cellulose papers were applied onto the eye so that one half of the paper covered the cornea and the other half covered the conjunctiva (limbal straddling) (Fig 1). Gentle pressure was applied with the blunt edge of a forceps, and the strip was carefully peeled off the epithelial surface. All patients were told to bring one healthy accompanying person without any history of eye exposure or pathologies. The accompanying person's ocular surface health was confirmed with a careful history taking and complete ocular examination.

After sampling, the filter paper with the specimen was fixed in a cytology fixative containing glacial acetic acid, formaldehyde, distilled water, and ethyl alcohol in a 1:1:6:14 volume ratio and within a labeled 24-well container. In the pathology laboratory, the specimens were rehydrated in 70% alcohol and stained with a combination of Periodic acid-Schiff and Papanicolaou staining.<sup>10,21</sup> Simultaneously, one slide from a goblet cell containing conjunctiva as the positive control and one slide from the cornea as the negative control were stained from the healthy accompanying person. Finally, the papers were cleared in xylene and each paper was mounted with a DPX mountant (Depex-Polystyrene dissolved in xylene [BDH/Merck, Lutterworth, UK]) on a glass slide with the epithelial cells facing up. The prepared slides were examined with a light microscope (Olympus BX43, Tokyo, Japan) attached to a digital camera (Olympus DP12) by one ophthalmopathologist (MRK) who was unaware of the patient's clinical manifestations and history.

The presence of goblet cells on the corneal half of the specimen was considered as evidence of corneal conjunctivalization and LSCD. Goblet cells were counted in a selected microscopic field ( $\times 40$ ) with the highest density of goblet cells on the corneal side of each specimen. The values were graded as follows: 0 = absence of goblet cells, 1 = <10 goblet cells/field, 2 = 11–200 goblet cells/field, 3 = >200 goblet cells/field. To grade the degree of epithelial squamous metaplasia, we scored the cytopathologic and pyknotic changes, cytoplasm color, degree of keratinization, and presence of inflammatory cells (Table 1). Total points of 0 to 2, 3 to 5, 6 to 9, 10 to 11, and 12 were considered as no squamous metaplasia, mild, moderate, severe, and most severe squamous metaplasia, respectively. If the number of insufficient samples was

Table 1. Cytopathologic Criteria for Grading Squamous Metaplasia

Points	0	1	2	3
N/C ratio	1:2	1:4	1:8	1:12
Nucleus condensation	None	Few snakes and pyknosis	Moderate snakes and pyknosis	Extensive snakes and pyknosis
Inflammatory cell infiltrate	None	Mild	Moderate	Severe
Cytoplasm color and keratinization	Blue-green color	Pinkish (metachromatic), some cells contain keratin filaments	More cells contain densely packed keratin filaments	Shrunken densely packed keratin filaments

N/C ratio = nucleus/cytoplasm ratio.

more than 2, resampling was performed. The eyes with at least 3 of 5 sufficient samples were included.

## Results

Thirty-nine eyes of 20 patients (all male) were evaluated. Four eyes of 2 patients were excluded because of insufficient samples. Finally, 35 eyes of 18 patients were included. One patient had only 1 eye as the result of warfare trauma. The mean age of patients was  $44.5 \pm 3.2$  years (38–49 years) at the time of sampling. The mean time interval between mustard gas exposure and sampling was  $20 \pm 1.7$  years (18–24 years).

There were 6 (17.1%), 10 (28.6%), 6 (17.1%), 5 (14.3%), and 15 (42.9%) insufficient specimens in the superior, inferior, nasal, temporal, and central quadrants, respectively. Goblet cells were found in at least 1 quadrant of cornea in all 35 eyes (100% of cases). After excluding insufficient specimens, 13 (44.8%), 8 (32.00%), 15 (51.7%), 15 (50.0%), and 5 (25.0%) specimens were positive for goblet cells in the superior, inferior, nasal, temporal, and central quadrants of the corneas, respectively (Table 2). There was no difference between impression cytology findings (positive vs. negative for corneal goblet cells) among different quadrants ( $P = 0.378$ ). Grade 1 LSCD was found in 19 (65.5%), 15 (60.0%), 12 (41.4%), 11 (36.7%), and 17 (85.0%) specimens in the superior, inferior, nasal, temporal, and central quadrants, respectively. Grade 2 LSCD was found in 10 (34.5%), 10 (40.0%), 17 (58.6%), 19 (63.3%), and 3 (15.0%) specimens in the superior, inferior, nasal, temporal, and central quadrants, respectively. No cases of grade 3 LSCD were found in our samples. There was no difference in grading of LSCD among all quadrants ( $P = 0.734$ ).

There was no difference in clinical grading between nasal and temporal quadrants ( $P = 0.266$ ) and between superior and inferior quadrants ( $P = 0.263$ ) (Table 3). By combining superior and inferior quadrants (vertical zone) and nasal and temporal quadrants (horizontal zone), corneal clinical grading was more severe in horizontal versus vertical zones ( $P < 0.001$ ). With this sample size,

combining all quadrants without considering their locations, there was no relation between impression cytology findings (positive vs. negative for goblet cells) and corneal clinical grading ( $P = 0.893$ ).

Squamous cell metaplasia was one of the important cytopathologic features in our study (Table 2). Severe squamous metaplasia was noted in only 2 (6.67%) temporal quadrants. There was no relation between severity of squamous metaplasia and presence of goblet cells on the corneal surface ( $P = 0.727$ ). Corneal clinical manifestations also were not related to the degree of squamous metaplasia ( $P = 0.917$ ).

## Case Reports

**Case 5.** A 42-year-old man who was exposed to mustard gas 21 years ago presented with red eyes, severe photophobia, blepharospasm, foreign body sensation, and visual acuity loss in both eyes. Uncorrected visual acuity was 20/200 and 20/160 in the right and left eyes, respectively. On biomicroscopic examination, there was thinning and lipoid/amyloid deposition in the nasal and temporal quadrants, and neovascularization in the superior and nasal quadrants of the right cornea (Fig 2A). There was also severe thinning and lipoid/amyloid deposition in the superior, temporal, and nasal quadrants of the left cornea. Neovascularization was evident in the superior and nasal quadrants (Fig 2B). Impression cytology showed goblet cells in the inferior, nasal, and temporal quadrants of the right eye and in the superior, inferior, and central quadrants of the left eye (Fig 2C).

**Case 12.** A 48-year-old man with a history of exposure to mustard gas 21 years ago was referred with foreign body sensation, photophobia, and reduction in visual acuity in both eyes. Uncorrected visual acuity was 20/80 and 20/160 in the right and left eyes, respectively. Biomicroscopic examination revealed severe thinning in the temporal quadrant of the right eye associated with amyloid deposition and invading telangiectatic vessels (Fig 3A). In the left eye, there was also thinning in the temporal quadrant with lipoid/amyloid deposition and invading telangiectatic vessels extending to the inferior quadrant (Fig 3B). Impression cytology

Table 2. Impression Cytology Data

Quadrant	Goblet Cell Positive (%)	Goblet Cell Negative (%)	Squamous Metaplasia (%)			
			Not Seen	Mild	Moderate	Severe
Superior	13 (44.83)	16 (55.18)	10 (34.48)	10 (34.48)	9 (31.03)	0 (0.00)
Inferior	8 (32.00)	17 (68.00)	8 (32.00)	7 (28.00)	10 (40.00)	0 (0.00)
Nasal	15 (51.72)	14 (48.27)	15 (51.72)	11 (37.93)	3 (10.34)	0 (0.00)
Temporal	15 (50.00)	15 (50.00)	7 (23.33)	13 (43.33)	6 (20.00)	2 (6.67)
Central	5 (25.00)	15 (75.00)	5 (25.00)	9 (45.00)	6 (30.00)	0 (0.00)

Percentages were calculated after excluding insufficient specimens.

Table 3. Clinical Grading of Severity in Different Corneal Quadrants

Quadrant	Superior	Inferior	Nasal	Temporal	Central
Grade					
Not seen	8 (22.9%)	9 (25.7%)	3 (8.6%)	3 (8.6%)	9 (25.7%)
Mild	16 (45.7%)	14 (40.0%)	9 (25.7%)	6 (17.1%)	13 (37.1%)
Moderate	10 (28.6%)	9 (25.7%)	14 (40.0%)	15 (42.9%)	8 (22.9%)
Severe	1 (2.9%)	3 (8.6%)	9 (25.7%)	11 (31.4%)	5 (14.3%)

confirmed LSCD in the inferior, nasal, and temporal quadrants of the right eye and in the superior, temporal, and central quadrants of the left eye (Fig 3C).

## Discussion

Our results show that there is LSCD in all patients with chronic or delayed-onset MGK confirmed by impression cytology. Limbal stem cell deficiency was mild to moderate in all of our cases. There was no relation between corneal clinical manifestations and LSCD. Although LSCD could be seen in all affected quadrants, corneal clinical manifestations were more severe in the temporal and nasal quadrants.

The moist ocular surface and extreme lipophilic nature of the gas combine to increase its affinity for the lipid layer of the tear film, making the eye the most susceptible part of the body.<sup>17,22</sup> Corneal epithelial cells are extremely susceptible because of their rapid turnover, high metabolic rate, and prolonged interaction with the agent that concentrates in the oily tear layer.<sup>17</sup> Varying degrees of ocular involvement are seen in 75% to 90% of individuals who are exposed to mustard gas in the acute stage. They may heal completely or progress to chronic or delayed-onset forms, which are almost always progressive and may cause irreversible damage to the eye and result in blindness.<sup>13</sup>

Despite detecting goblet cells on the cornea demonstrating LSCD, presentation of conjunctivalization is not typical in these cases. Progressive wave of conjunctivalization and late fluorescein staining are difficult to detect because of multiple recurrent and sometimes persistent corneal epithelial defects, corneal thinning in several areas, lipid and amyloid deposition, and several areas of fluorescein staining and pooling. Moreover, a total vascularized pannus could not be seen; instead, telangiectatic, leaking, aberrant, tortuous vessels invade the peripheral cornea. Corneal manifestations in patients with MGK also are not typical for LSCD, as it could be seen in severe chemical burns. Distinctive corneal features common to most cases include epithelial irregularity, recurrent or persistent epithelial defects, neovascularization, stromal scarring, thinning, melting, and secondary degenerative changes, including lipid and amyloid deposition.<sup>13</sup>

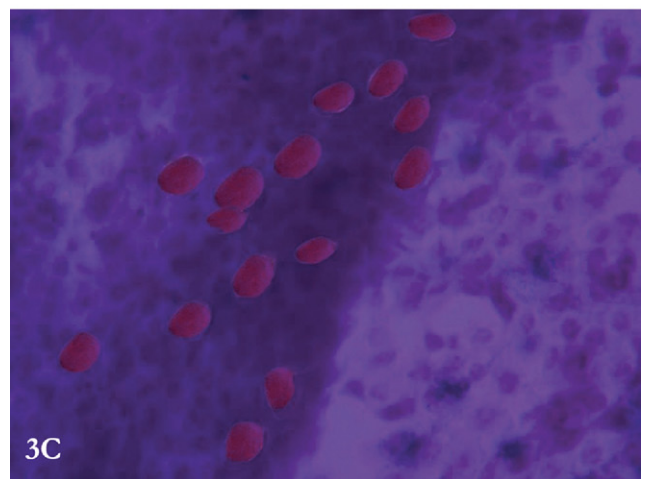
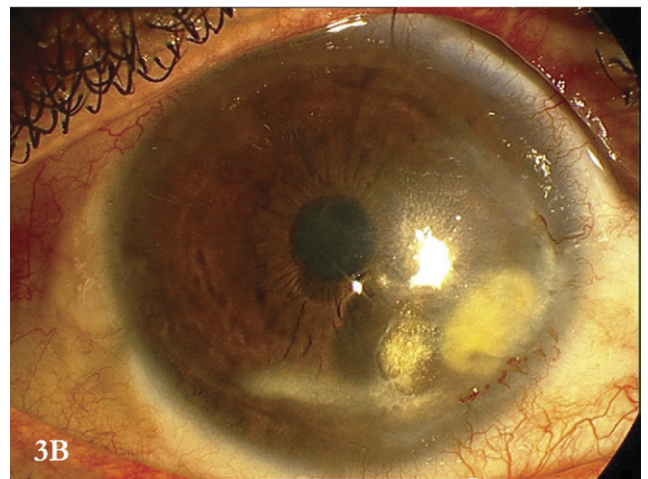
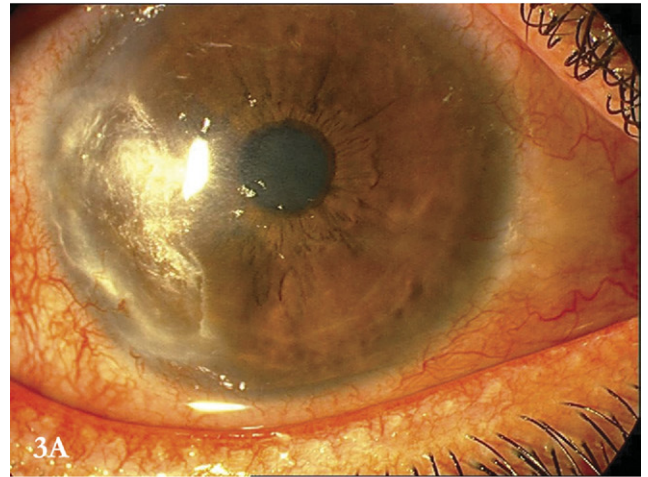
It seems that late clinical manifestations of MGK are the result of 3 underlying mechanisms: (1) Progressive LSCD, which at the beginning is partial and asymmetric and finally causes total LSCD. This may be a result of the direct and progressive detrimental effect of mustard gas or a consequence of chronic limbal ischemia.<sup>13,19</sup> (2) Chronic, progressive, and severe perilimbal conjunctival ischemia, which is

mainly seen in the palpebral fissure area. This may lead to stromal thinning and epithelial disintegrity of the adjacent cornea. These areas of conjunctival ischemia are often surrounded by invading leaking telangiectatic vessels leading to lipid depositions in the adjacent cornea.<sup>13,17,19</sup> (3) Lipoid deposition resulting from invading telangiectatic vessels triggering chronic stromal inflammation and thinning. This may aggravate the clinical situation and produce severe photophobia and tearing.<sup>23</sup> Limbal ischemia may play a significant role in delayed MGK. It can chronically and progressively lead to corneal neurotrophic and trophic changes, including thinning, descemetocele formation, and perforation. Furthermore, it may progressively destroy the limbal stem cells and stromal niche, which may expedite the process of progressive LSCD.

Corneal manifestations are more severe in exposure zones, especially in the nasal and temporal quadrants. These may be caused by direct exposure of interpalpebral fissure during the time of exposure. Furthermore, perilimbal ischemia is more severe in these areas, which also may be caused by direct exposure of tissues with consequent vasculitis. Moreover, some authors have suggested that there are fewer stem cells in the nasal and temporal quadrants, which can potentially put these regions at higher risk.<sup>24–27</sup>

In previous studies,<sup>13,18</sup> histopathologic features of a conjunctival component in patients with MGK included chronic inflammation, perilimbal conjunctival ischemia, telangiectasis, vasculitis, subconjunctival hemorrhage, decreased number of goblet cells, thinning or thickening of epithelium, scar formation in substantia propria associated with lymphocytic infiltration, and dilated lymphatic vessels. There were no signs of dysplasia. Corneal pathologic findings included destruction of the epithelium and Bowman's layer, loss of keratocytes, conjunctivalization, superficial and stromal vascularization, squamous metaplasia, focal corneal thinning, ulceration, mild to moderate acute and chronic infiltration of inflammatory cells, lipid/amyloid deposition, endothelial cell loss, calcific band keratopathy, and scarring in the stroma.<sup>13,18</sup>

Squamous metaplasia is a reversible adaptive phenomenon in some epithelia, including ocular surface epithelium against external pathogenic stimuli. Determination of the grades of metaplasia is an important factor in evaluating the severity of ocular surface disorder.<sup>28,29</sup> More recently, impression cytology has been used to demonstrate squamous metaplasia as a diagnostic factor in cases with LSCD.<sup>28</sup> In the current study, squamous metaplasia was not observed in approximately one third to one half (range, 23.3%–51.72%) of the specimens from all quadrants. Squamous metaplasia was mild to moderate in most specimens. Lopez-Garcia et



**Figure 2.** Biomicroscopic examination shows (A) thinning and lipid/amyloid deposition in the nasal and temporal quadrants, neovascularization in the superior and nasal quadrants of the right cornea, and (B) severe thinning, lipid/amyloid deposition in the superior, temporal, and nasal quadrants. Neovascularization is evident in the superior and nasal quadrants of the left cornea. C, Impression cytology shows goblet cells in both corneas confirming LSCD.

**Figure 3.** Biomicroscopic examination shows (A) severe thinning in the temporal quadrant of the right eye associated with amyloid deposition and telangiectatic vessels in the adjacent cornea and (B) thinning in the temporal quadrant with lipid/amyloid deposition and telangiectatic vessels in the adjacent cornea extending to the inferior quadrant of the left eye. C, Impression cytology shows goblet cells in both corneas confirming LSCD.

al<sup>29</sup> suggested that corneal epithelial squamous metaplasia may be a diagnostic factor in cases with mild or subclinical limbal deficiency. In our series, corneal epithelial squamous metaplasia may be a reactive response to mustard gas exposure and its late consequences. We observed severe squamous metaplasia in 2 specimens from the temporal quadrant, where the corneal manifestations, perilimbal ischemia, and vasculitis seem to be more severe. Squamous metaplasia may occur in lesser or greater degrees parallel to other features seen in MGK, such as dry eye, LSCD, and perilimbal ischemia. No significant relationship was observed between the corneal clinical manifestations and the degree of squamous metaplasia. Perilimbal conjunctival vasculitis and ischemia might be assumed as factors intensifying corneal clinical manifestations with possible subtle effects on corneal cytopathologic features.

Because of severe photophobia and tearing, and despite decreasing sampling room luminance and applying anesthetic drop, obtaining corneal samples for impression cytology was difficult in these patients. We repeated impression cytology once in some of our cases with insufficient samples to obtain adequate specimens. Despite this, 4 eyes of 2 patients were excluded and 42 samples of included eyes were insufficient for evaluation. The inferior and central quadrants had the maximum percentage of insufficient specimens, which may be due to accumulation of tear for the inferior quadrant and prolongation of obtaining specimens for the central quadrant. There were some quadrants without goblet cells, which may be the result of excessive tearing washing out the samples in the advanced stages of disease. Furthermore, stromal thinning-melting, epithelial irregularity, and amyloid/lipoid deposition are more severe in the nasal and temporal quadrants, which may also affect our sampling.

One important possibility is that LSCD may be more severe in the nasal and temporal quadrants, similar to corneal clinical manifestations. Because of the semiquantitative nature of the evaluation, limited number of cases, consecutive inclusion, different clinical stages, excessive tearing, and surface irregularities in the exposure zones, this concern was not definitively addressed.

## Limitations

There are some limitations in our study. Forty-two (24.0%) of 175 samples did not yield sufficient cells to interpret. This may affect our results. Our patients were consecutively included. They were obviously in different stages of ocular involvement, especially in the moderate and severe stages, and were seeking medical advice. Therefore, our results may not be generalized to different clinical stages of MGK.

In conclusion, our results confirmed LSCD in patients with chronic or delayed MGK. Corneal clinical manifestations are more severe in the nasal and temporal quadrants. No relation between LSCD and clinical manifestations was observed. Other factors, such as perilimbal conjunctival ischemia, may play a role.

## References

1. Tseng SC. Concept and application of limbal stem cells. *Eye* 1989;3:141–57.
2. Tseng SC. Regulation and clinical implications of corneal epithelial stem cells. *Mol Biol Rep* 1996;23:47–58.
3. Dua HS, Azuara-Blanco A. Limbal stem cells of the corneal epithelium. *Surv Ophthalmol* 2000;44:415–25.
4. Lavker RM, Tseng SC, Sun TT. Corneal epithelial stem cells at the limbus: looking at some old problems from a new angle. *Exp Eye Res* 2004;78:433–46.
5. Hatch KM, Dana R. The structure and function of the limbal stem cell and the disease states associated with limbal stem cell deficiency. *Int Ophthalmol Clin* 2009;49:43–52.
6. Li W, Hayashida Y, Chen YT, Tseng SC. Niche regulation of corneal epithelial stem cells at the limbus. *Cell Res* 2007;17:26–36.
7. Dua HS, Saini JS, Azuara-Blanco A, Gupta P. Limbal stem cell deficiency: concept, aetiology, clinical presentation, diagnosis and management. *Indian J Ophthalmol* 2000;48:83–92.
8. Dua HS, Joseph A, Shanmuganathan VA, Jones RE. Stem cell differentiation and the effects of deficiency. *Eye* 2003;17:877–85.
9. Espana EM, Di Pascuale M, Grueterich M, et al. Keratolimbal allograft in corneal reconstruction. *Eye* 2004;18:406–17.
10. Puangsrichareon V, Tseng SC. Cytologic evidence of corneal diseases with limbal stem cell deficiency. *Ophthalmology* 1995;102:1476–85.
11. McKelvie P. Ocular surface impression cytology. *Adv Anat Pathol* 2003;10:328–37.
12. Ghasemi H, Ghazanfari T, Babaei M, et al. Long-term ocular complications of sulfur mustard in the civilian victims of Sardasht, Iran. *Cutan Ocul Toxicol* 2008;27:317–26.
13. Javadi MA, Yazdani S, Sajjadi H, et al. Chronic and delayed-onset mustard gas keratitis: report of 48 patients and review of literature. *Ophthalmology* 2005;112:617–25.
14. Safarinejad MR, Moosavi SA, Montazeri B. Ocular injuries caused by mustard gas: diagnosis, treatment, and medical defense. *Mil Med* 2001;166:67–70.
15. Mann I. A study of eighty-four cases of delayed mustard gas keratitis fitted with contact lenses. *Br J Ophthalmol* 1944;28:441–7.
16. Banin E, Morad Y, Berenshtein E, et al. Injury induced by chemical warfare agents: characterization and treatment of ocular tissues exposed to nitrogen mustard. *Invest Ophthalmol Vis Sci* 2003;44:2966–72.
17. Solberg Y, Alcalay M, Belkin M. Ocular injury by mustard gas. *Surv Ophthalmol* 1997;41:461–6.
18. Pleyer U, Sherif Z, Baatz H, Hartmann C. Delayed mustard gas keratopathy: clinical findings and confocal microscopy. *Am J Ophthalmol* 1999;128:506–7.
19. Javadi MA, Baradaran-Rafii A. Living-related conjunctival-limbal allograft for chronic or delayed-onset mustard gas keratopathy. *Cornea* 2009;28:51–7.
20. Javadi MA, Yazdani S, Kanavi MR, et al. Long-term outcomes of penetrating keratoplasty in chronic and delayed mustard gas keratitis. *Cornea* 2007;26:1074–8.
21. Tseng SC. Staging of conjunctival squamous metaplasia by impression cytology. *Ophthalmology* 1985;92:728–33.
22. Aasted A, Darre E, Wulf HC. Mustard gas: clinical, toxicological, and mutagenic aspects based on modern experience. *Ann Plast Surg* 1987;19:330–3.
23. Mann I, Pullinger BD. The pathology of cholesterol and fat deposition in mustard gas injuries of the cornea. *Br J Ophthalmol* 1942;26:503–7.

24. Kwok LS, Coroneo MT. A model for pterygium formation. *Cornea* 1994;13:219–24.
25. Pajooohesh-Ganji A, Ghosh SP, Stepp MA. Regional distribution of alpha9beta1 integrin within the limbus of the mouse ocular surface. *Dev Dyn* 2004;230:518–28.
26. Pajooohesh-Ganji A, Pal-Ghosh S, Simmens SJ, Stepp MA. Integrins in slow-cycling corneal epithelial cells at the limbus in the mouse. *Stem Cells* 2006;24:1075–86.
27. Zhao J, Mo V, Nagasaki T. Distribution of label-retaining cells in the limbal epithelium of a mouse eye. *J Histochem Cytochem* 2009;57:177–85.
28. Singh R, Joseph A, Umopathy T, et al. Impression cytology of the ocular surface. *Br J Ophthalmol* 2005;89:1655–9.
29. Lopez-Garcia JS, Rivas L, Garcia-Lozano I. Corneal epithelium squamous metaplasia determination as diagnostic factor in limbal deficiency [in Spanish]. *Arch Soc Esp Oftalmol* 2006;81:281–8.

## Footnotes and Financial Disclosures

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