

Inflammatory cytokine and osmolarity
changes in the tears of dry eye patients
treated with topical 1%
methylprednisolone

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Directed by Professor Tae-im Kim

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This certifies that the Master's Thesis of
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ABSTRACT

Inflammatory cytokine and osmolarity changes in the tears of dry eye patients treated with topical 1% methylprednisolone

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Dry eye syndrome is a common condition, affecting approximately 10-20% of the adult population, and its pathogenesis has not been clearly established. However, there is increasing evidences that ocular inflammation plays a key role in the pathogenesis of dry eye syndrome. This dissertation deals with changes in clinical outcome, inflammatory cytokine levels, and tear osmolarity in the tears of patients with dry eye syndrome both before and after the application of topical 1% methylprednisolone.

Thirty-two patients (64 eyes) with moderate to severe dry eye were enrolled. Topical 1% methylprednisolone was applied four times per day for 1 month and tapered for the next month. Preservative-free 0.1% sodium hyaluronate was also applied 4-6 times per day. Corneal and conjunctival staining scores, tear film breakup time (TFBUT), and tear osmolarity were assessed at baseline, week 4, and week 8. Tear samples were collected at every visit for cytokine analysis.

Corneal and conjunctival staining scores and TFBUT showed statistically significant improvement at weeks 4 and 8. Schirmer test scores were improved at weeks 4 and 8, but there were no statistically significant differences. Tear osmolarity decreased significantly at week 8. All cytokine levels decreased at weeks 4 and 8, and interleukin (IL)-1 β , IL-8, IL-17, and monocyte chemoattractant protein-1 were significantly decreased at week 8 compared with baseline. No adverse events were observed during the study period.

Short-term treatment with topical 1% methylprednisolone not only improved clinical outcome but also decreased tear osmolarity and inflammatory cytokine levels without significant adverse events. Measurement of inflammatory cytokine levels and tear osmolarity, in addition to widely used clinical parameters, might be used as an

objective method to evaluate the efficacy of treatment in dry eye patients.

Key words: dry eye, inflammatory cytokine, osmolarity, 1% methylprednisolone

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I. INTRODUCTION

Dry eye syndrome is a common condition, affecting approximately 10-20% of the adult population,¹ and is associated with subjective symptoms including ocular discomfort, visual disturbance, dryness, and soreness.^{2,3} Objective signs including tear film instability and ocular surface inflammation often accompany these symptoms.³

The pathogenesis of dry eye syndrome has not been clearly established. However, there is increasing evidence that ocular surface inflammation plays a key role in the pathogenesis of dry eye. Increased levels of inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF- α), and chemokines such as IL-8, CXC chemokine ligand 9 (CXCL9), CXCL10, and CXCL11, have been detected in the tears of dry eye patients.⁴⁻⁶

Immunopathological changes have also been detected in the conjunctiva of dry eye syndrome patients, including increased expression of major histocompatibility class II human leukocyte antigen-DR and intercellular adhesion molecule 1, and increased infiltration of T lymphocytes.⁷

Clinical evidence has shown that anti-inflammatory treatments such as topical corticosteroids and cyclosporine are effective in the treatment of dry eye syndrome.⁸⁻¹⁰ Topical methylprednisolone has been reported to reduce the expression of matrix metalloproteinase as well as levels of inflammatory cytokines in experimental murine dry eye.¹¹

In previous studies, tear osmolarity correlated significantly with dry eye severity grade¹² and was found to be the best marker of disease severity.¹³ With recent technological advances, minimally invasive measurements of tear osmolarity have become available.

Although increased inflammatory cytokine levels and osmolarity in the tears of

dry eye syndrome patients were confirmed by previous studies,^{4-6,12,13} there have not been studies that investigate changes in inflammatory cytokine levels and tear osmolarity both before and after treatment. The purpose of this study is to determine whether there are differences in tear cytokine levels and osmolarity both before and after treatment with topical 1% methylprednisolone and preservative-free artificial tears.

II. MATERIALS AND METHODS

1. Patient Selection

Thirty-two patients (64 eyes) with moderate to severe dry eye whose symptoms and signs were unresponsive to aqueous enhancement therapy were enrolled in this study. Inclusion criteria were as follows: tear film breakup time (TFBUT) less than 5 seconds and positive corneal and conjunctival staining. Exclusion criteria included the presence of any ocular disease other than dry eye syndrome, history of ocular surgery, use of contact lenses, use of other topical ocular medications, and history of hypersensitivity or adverse events to the study medication. The study was approved by the Institutional Review Board of

Severance Hospital and conducted according to the Declaration of Helsinki and Good Clinical Practices. Informed consent was obtained from all patients.

2. Study Design

At the initial visit, patients were assessed for whether they met the previously outlined inclusion and exclusion criteria, and the following parameters were also assessed: corneal and conjunctival fluorescein staining, TFBUT, tear osmolarity, and tear collection for cytokine analysis. All of these parameters were evaluated at baseline, week 4, and week 8.

Patients were instructed to apply topical 1% methylprednisolone four times per day for the first 4 weeks and were re-evaluated at week 4. Based on their symptoms and signs, the methylprednisolone eyedrops were continued or gradually applied at a lower frequency. Preservative-free 0.1% sodium hyaluronate was also applied 4-6 times per day during the study period.

All examinations were performed and recorded by the same examiner, and safety was assessed by monitoring any adverse events throughout the duration of the study.

3. Study Materials

Topical methylprednisolone was prepared by diluting intravenous methylprednisolone sodium succinate in non-preserved sterile normal saline at a final concentration of 1%.⁸ Patients were instructed to keep the steroid solution refrigerated and to discard it after 4 weeks. Preservative-free 0.1% sodium hyaluronate (Kynex, Alcon Laboratory, Seoul, Korea) was used in this study.

4. Clinical Assessment

A. Corneal Staining

The degree of staining was measured for each of the five regions of the cornea: central, superior, temporal, nasal, and inferior. The degree of staining was based on the following: grade 0 (normal): no staining; grade 1 (mild): superficial stippling and micropunctate staining; grade 2 (moderate): macropunctate staining with some coalescent areas; and grade 3 (severe): numerous coalescent macropunctate areas and/or patches. Each of the five regions was graded on a scale from 0 to 3. The maximum score for each area was 3. The scores of the five areas were added to obtain a total score for each eye (the maximum score

for each eye was 15).

B. Conjunctival Staining

The degree of staining was separately assessed for the three portions of the temporal conjunctiva and the three portions of the nasal conjunctiva on a scale from 0 to 3 (the maximum score for each area is 3). The scores for each of the six areas were added to obtain a total score for each eye (the maximum score for each eye was 18).

C. TFBUT and Schirmer test

The investigator monitored the integrity of the tear film and measured the time from the last blink to the point where one or more dry spots appeared in the precorneal tear film. Schirmer II test with topical anesthesia was performed to evaluate the basal tear secretion.

D. Tear Osmolarity

Tear osmolarity was measured using a handheld TearLabTM device (TearLab Co.,

San Diego, CA, USA). 50 nL tear sample was obtained from the inferior tear lake near the lateral canthus and was collected at the bottom tip of the test card. An analysis was performed using a disposable lab-on-a-chip, and a read-out was quickly generated by a separate desktop machine.

5. Tear Collection and Multiplex Immunobead Assay

30 μ L of phosphate-buffered saline was instilled into the conjunctival sac. A 20 μ L volume of tear fluid and buffer were collected with a micropipette at the lateral canthus, avoiding an additional tear reflex. The fluid was placed into a 1.5 mL Eppendorf tube, which was stored at -70°C until further examination.

Cytokine concentrations were measured using multiplex immunobead assay (BDTM Cytometric Bead Array Human Soluble Protein Flex Set, BD Biosciences, San Jose, CA, USA) and flow cytometry (BDTM FACS LSR II, BD Biosciences, San Jose, CA, USA). Cytokines and chemokines analyzed included IL-1 β , IL-6, IL-17, interferon gamma (IFN- γ), TNF- α , IL-8, and monocyte chemotactic protein-1 (MCP-1).

6. Safety Monitoring

All adverse events that could arise during the course of the study, including elevation of intraocular pressure, were assessed by the examiner using slit lamp examination and tonometry using a pneumatic tonometer at every visit. Patients were also encouraged to report any unfavorable or unintended symptoms or signs such as a decrease of vision, hyperemia, and ocular pain.

7. Statistical Analysis

Changes in clinical parameters, tear osmolarity, and cytokine levels both before and after treatment were compared using a paired t-test. Dry eye signs, tear osmolarity, and inflammatory cytokine concentrations at week 4 and week 8 were compared with the baseline values. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

III. RESULTS

1. Patients

Thirty-two patients (64 eyes) were enrolled in this study, but five patients were lost to follow up after their baseline visit. Twenty-seven patients (54 eyes) who completed the study were eligible for analysis.

Mean values at baseline are presented in Table 1. As inclusion criteria included TFBUT less than 5 seconds and positive corneal and conjunctival staining, mean corneal staining score and conjunctival staining score were 3.14 ± 2.08 and 2.06 ± 1.06 , respectively. Mean TFBUT and Schirmer test score were 4.15 ± 1.64 seconds and 8.67 ± 3.46 , respectively. Mean tear osmolarity at baseline (309.31 ± 17.26 mOsm/L) was similar to that found by Suzuki et al. (309.7 ± 22.3 mOsm/L).¹² Seven cytokines were detected; IL-8 (106.22 ± 134.02 pg/mL) and MCP-1 (42.41 ± 80.84 pg/mL) were highly elevated.

TABLE 1. Descriptive Statistics for Signs, Tear Osmolarity, and Inflammatory Cytokines in Dry Eye Patients

	Mean	Median
Signs		
Corneal staining score	3.14 ± 2.08	3.00
Conjunctival staining score	2.06 ± 1.06	2.00
TFBUT	4.15 ± 1.64	3.00
Schirmer test	8.67 ± 3.46	8.00
Tear osmolarity	309.31 ± 17.26	308.50
Concentration of inflammatory cytokines		
IL-1 β	2.13 ± 2.95	1.22
IL-6	5.15 ± 16.60	0.00
IL-8	106.22 ± 134.02	41.91
IL-17	1.22 ± 2.53	0.00
IFN- γ	0.38 ± 1.12	0.00
TNF- α	0.17 ± 0.73	0.00
MCP-1	42.41 ± 80.84	12.32

2. Changes of Dry Eye Signs and Tear Osmolarity

Corneal staining score, conjunctival staining score, and TFBUT showed statistically significant improvement at week 4 and 8 compared with baseline. Schirmer test scores were increased at week 4 and 8, but there were no statistically significant differences. A statistically significant decrease in tear osmolarity was observed at week 8 compared with baseline ($p=0.003$). Tear osmolarity decreased at week 4 and 8, which was statistically significant at week 8 (Table 2, Figure 1).

TABLE 2. Changes in Dry Eye Signs and Tear Osmolarity

	Baseline	4 weeks	p-value*	8 weeks	p-value*
Corneal staining score	3.14 ± 2.08	1.63 ± 1.54	<0.001	0.97 ± 1.07	<0.001
Conjunctival staining score	2.06 ± 1.06	1.33 ± 1.35	<0.001	0.65 ± 1.02	<0.001
TFBUT	4.15 ± 1.64	5.74 ± 1.53	<0.001	7.09 ± 2.14	<0.001
Schirmer test	8.67 ± 3.46	9.00 ± 3.85	0.942	9.21 ± 4.73	0.598
Tear osmolarity	309.31 ± 17.26	306.98 ± 16.07	0.990	301.18 ± 15.15	0.003

* paired t-test was used.

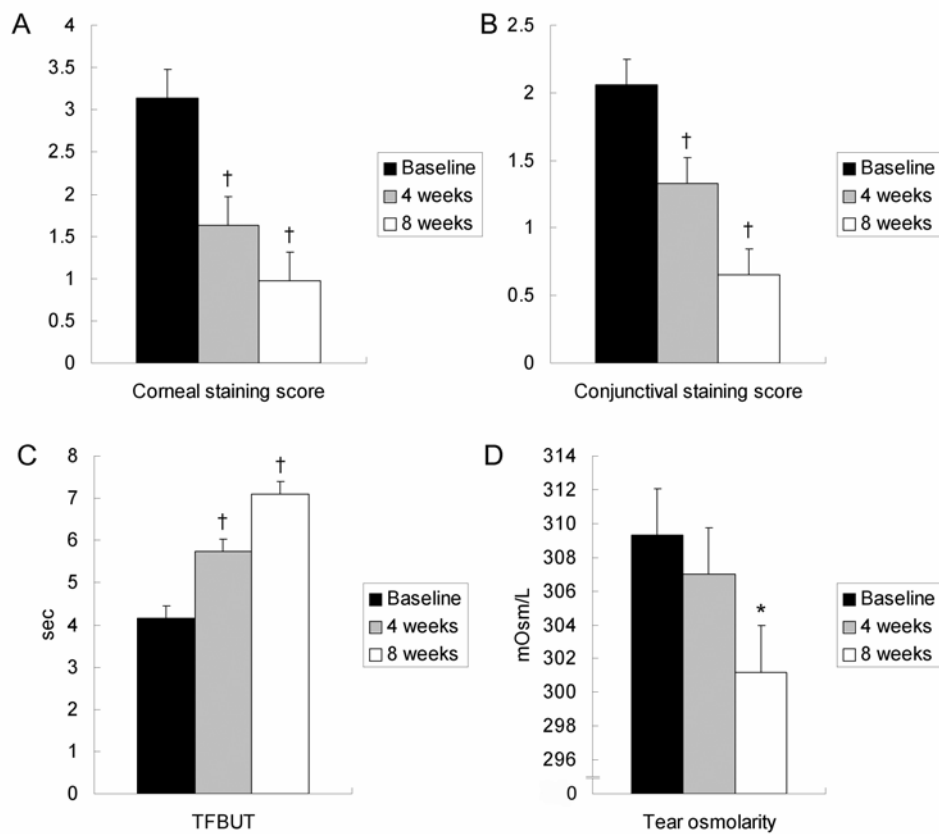


FIGURE 1. Changes in corneal staining score (A), conjunctival staining score (B), TFBUT (C), and tear osmolarity (D). * $p < 0.01$, $^{\dagger}p < 0.001$.

3. Changes in Inflammatory Cytokine Concentrations

IL-1 β , IL-8, IL-17, and MCP-1 were significantly decreased at week 8 compared with baseline ($p=0.004$, 0.001 , 0.039 , 0.032 respectively). The concentrations of these cytokines also decreased at week 4, but the differences were not statistically significant. Other cytokines (IL-6, IFN- γ , and TNF- α) also decreased at week 4 and 8 but was not statistically significant (Table 3, Figure 2).

TABLE 3. Changes in Inflammatory Cytokine Concentrations

	Baseline	4 weeks	p-value*	8 weeks	p-value*
IL-1 β	2.13 $\pm$ 2.95	0.95 $\pm$ 1.92	0.096	1.30 $\pm$ 0.22	0.004
IL-6	5.15 $\pm$ 16.60	1.63 $\pm$ 4.78	0.264	0.16 $\pm$ 0.56	0.081
IL-8	106.22 $\pm$ 134.02	66.68 $\pm$ 143.33	0.444	26.44 $\pm$ 30.78	0.001
IL-17	1.22 $\pm$ 2.53	0.47 $\pm$ 1.38	0.221	0.43 $\pm$ 1.02	0.039
IFN- γ	0.38 $\pm$ 1.12	0.35 $\pm$ 0.99	0.833	0.37 $\pm$ 1.12	0.966
TNF- α	0.17 $\pm$ 0.73	0.09 $\pm$ 0.54	0.903	0.12 $\pm$ 0.50	0.732
MCP-1	42.41 $\pm$ 80.84	22.24 $\pm$ 28.61	0.155	11.29 $\pm$ 14.40	0.032

* paired t-test was used.

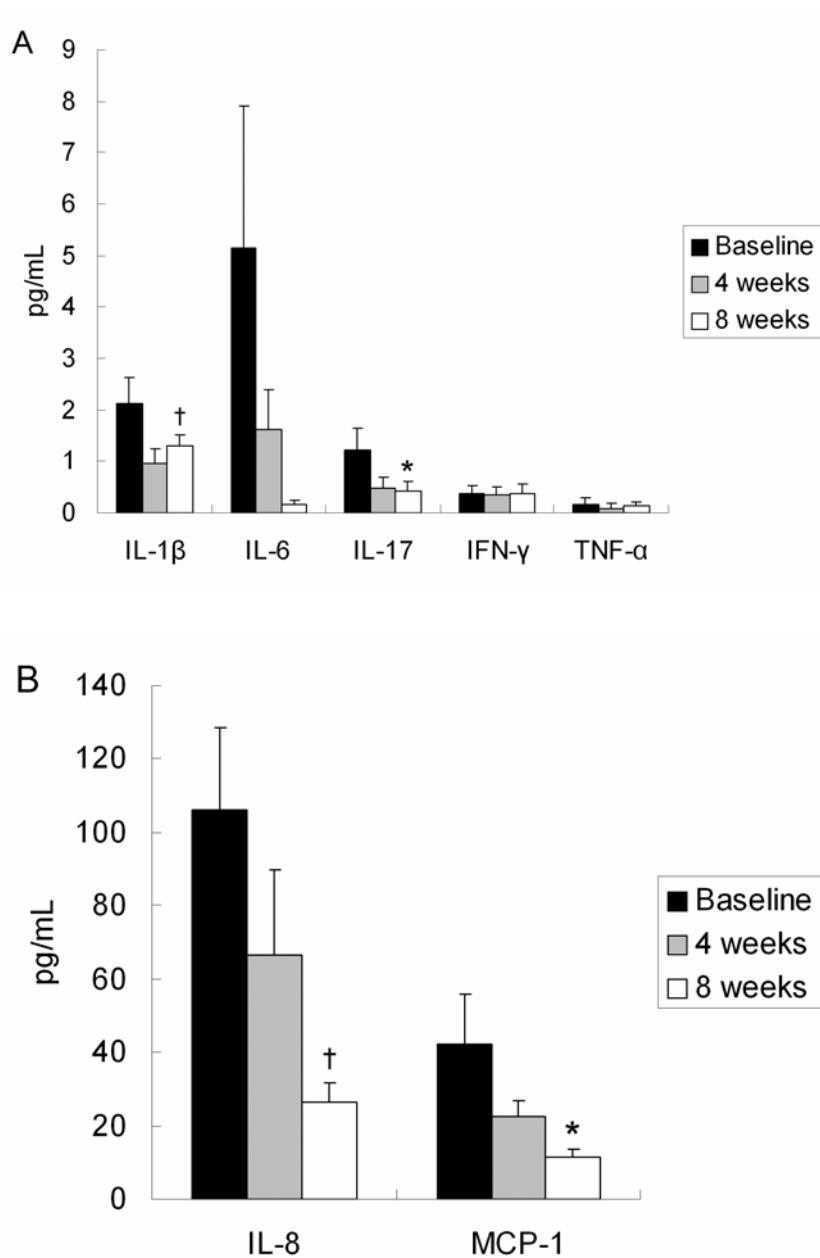


FIGURE 2. Changes in inflammatory cytokine concentrations. IL-1 β , IL-6, IL-17, IFN- γ , and TNF- α (A), IL-8 and MCP-1 (B). * $p < 0.05$, $^{\dagger}p < 0.01$.

4. Safety Results

No adverse events were observed by the examiner or reported by the patients during the study period; the study medications were well tolerated.

IV. DISCUSSION

This study showed that treatment with topical 1% methylprednisolone not only improves clinical indices, such as corneal staining score, conjunctival staining score, and TFBUT, but also decreases tear osmolarity and levels of inflammatory cytokines.

Tear hyperosmolarity is regarded as the central causing ocular surface inflammation, damage, and symptoms in dry eye syndrome.³ Hyperosmolarity stimulates a cascade of inflammatory events in the epithelial surface cells, involving mitogen-activated protein kinases (MAPKs), nuclear factor-kappa B signaling pathway,¹⁴ and the generation of inflammatory cytokines (IL-1 α , IL-1 β , and TNF- α) and MMP-9,¹¹ which activate inflammatory cells at the ocular surface.¹⁵ Increased levels of multiple pro-inflammatory cytokines in the tears of patients with dry eye syndrome, including IL-1, IL-6, IL-8, and TNF- α , have

been confirmed by previous studies.⁴⁻⁶

As it is not correlated to subsets of dry eye syndrome, tear osmolarity is believed to be a global indicator of the disease³ and has been proposed as the gold standard for diagnosis of dry eye.¹⁶ Previously, measurement of tear osmolarity was thought to require at least 5 μ L of tear sample, not practical for use in a clinical setting.¹⁷ It has also been difficult to determine tear osmolarity accurately due to reflex tearing.¹⁸ With technological advances, it is now possible to measure osmolarity using smaller amounts of tears (e.g., 50 nL in this study) and more conveniently by collecting tear samples from the inferior tear lake with the tip of the test card. Recent studies showed significant correlations between tear osmolarity and dry eye severity.^{12,13}

It is unique in this study that we proposed a possible objective approach to evaluate the efficacy of topical methylprednisolone in the treatment of patients with dry eye syndrome. Recent studies have shown that inflammatory cytokines and tear osmolarity are increased in dry eye syndrome and that tear osmolarity has a significant correlation with dry eye severity.^{4-6,12,13} It is anticipated that improvements in dry eye syndrome will result in a decrease in inflammatory cytokine levels and tear osmolarity. By measuring the changes in inflammatory cytokine levels and tear osmolarity, we could objectively confirm a decrease in tear osmolarity resulting from the anti-inflammatory effect of

methylprednisolone.

Methylprednisolone has been shown to have great efficacy in preserving corneal epithelial barrier function. De Paiva et al.¹¹ reported that methylprednisolone decreased levels of MMP-9, IL-1 α , IL-1 β , and TNF- α transcripts as well as activation of MAPKs. MMP-9 plays a key role in corneal barrier disruption in dry eye via the lysis of tight junction proteins in the apical epithelium, and the therapeutic effect of methylprednisolone may be primarily the result of MMP-9 inhibition.¹⁹ Moreover, methylprednisolone might secondarily inhibit MAPKs by decreasing the production of IL-1 and TNF- α that activate MAPKs, as corticosteroids have not been recognized as direct MAPKs inhibitors.^{11,20} In this study, we confirmed a significant decrease in IL-1 β levels after treatment with topical methylprednisolone.

IL-17 is a potent pro-inflammatory cytokine, and interaction of IL-17 with its receptor evokes activation of IL-8, resulting in recruitment of neutrophils to the injury site.²¹ MCP-1 has been identified as a key molecule for the chemotaxis of monocytes to the site of inflammation.²² The results of this study showed that IL-17, IL-8, and MCP-1 levels were all significantly decreased by topical methylprednisolone. These findings suggest that topical methylprednisolone improved ocular surface inflammation by decreasing the recruitment of inflammatory cells.

Other pro-inflammatory cytokines including IL-6, IFN- γ , and TNF- α also decreased, but the differences were not statistically significant in this study. Further and additional studies are required to define the changes in inflammatory cytokines after treatment with topical methylprednisolone.

Tear hyperosmolarity arises in situations of low aqueous tear flow, excessive evaporation, or a combination of these events.³ A previous study demonstrated that patients with faster tear film thinning time may be more susceptible to evaporation of tear film, thus increasing tear film evaporation, which may lead to a more concentrated tear film and increased osmolarity.²³ In this study, the stability of tear film (i.e., TFBUT) was significantly improved at weeks 4 and 8, and tear osmolarity significantly decreased at week 8.

Preservatives in topical agents such as benzalkonium chloride excite inflammatory cell markers at the ocular surface, causing epithelial cell damage and a decrease in mucin expression in addition to any direct effect on goblet cells.³ In this study, topical methylprednisolone was prepared as a non-preserved aqueous solution to avoid the toxicity associated with benzalkonium chloride. It should be considered that preservative-free 0.1% sodium hyaluronate was also used in this study. As sodium hyaluronate has a huge capacity to bind water, with an affinity 1000-fold of its own weight,²⁴ it was used to retain the aqueous layer of tear film at the ocular surface. There may be an effect of sodium hyaluronate in addition

to the therapeutic effects of methylprednisolone.

V. CONCLUSION

In this study, we objectively evaluated the anti-inflammatory effects of topical methylprednisolone in the treatment of dry eye patients by measuring the changes in inflammatory cytokine levels and tear osmolarity. With more technological advances, the changes in tear inflammatory cytokine levels and osmolarity, in addition to the widely used clinical parameters such as corneal and conjunctival staining, TFBUT, and Schirmer test, could be used as objective methods to evaluate the efficacy of treatment in dry eye patients.

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ABSTRACT(IN KOREAN)

1% 메틸프레드니솔론 점안제로 치료받은 건성안 환자에서
염증성 싸이토카인 및 눈물내 오스몰 농도의 변화

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이 지 환

건성안은 흔한 질환으로서, 성인 인구에서 약 10-20% 정도의 유병률을 보이는 것으로 알려져 있다. 건성안의 병인은 아직 명확히 밝혀져 있지는 않지만, 안구 표면의 염증이 건성안의 병인에서 핵심적인 역할을 하는 것으로 추정되고 있다. 고농도의 삼투질 농도에 의해 각막 상피세포에서 염증성 반응이 유발되고, 염증성 매개물질이 눈물로 분비되게 된다. 실제로 임상적으로도 corticosteroid 점안제 사용이 건성안 환자의 치료에서 효과적이었다고 보고되었고, 메틸프레드니솔론 점안제가 matrix metalloproteinase의 발현을 감소시키고,

염증성 사이토카인을 감소시키는 것으로 보고되었다.

본 연구에서는 32명 (64안)의 중등도 이상의 건성안 환자를 대상으로 1% 메틸프레드니솔론 점안제를 하루 4회씩 2달간 점안하도록 하였다. 치료 전과 치료 2달 후, 각막 및 결막 형광염색정도, 눈물막 파괴시간, Schirmer 검사, 눈물내 사이토카인 농도, 눈물내 오스몰 농도를 각각 측정하였다. 눈물내 사이토카인 농도는 BDTM Cytometric Bead Array Human Soluble Protein Flex Set (BD Biosciences, San Jose, CA, USA)을 이용하여 IL-1 β , IL-6, IL-8, IL-17, TNF- α , IFN- γ , monocyte chemoattractant protein-1 (MCP-1)을 대상으로 측정하였고, 눈물내 오스몰 농도는 TearLabTM Osmolarity System (TearLab Co., San Diego, CA, USA)을 사용하여 50 nL의 눈물을 눈물막에서 채취하였다.

각막, 결막 형광염색점수와 눈물막 파괴시간은 치료 4주 및 8주 후 통계적으로 유의한 감소를 보였다. Schirmer 검사점수는 4주 및 8주에 호전을 보였으나 통계적으로 유의한 정도는 아니었으며, 눈물내 오스몰 농도는 8주 후 유의한 감소를 보였다. 측정한 모든 염증성 사이토카인은 4주와 8주 후 감소되었고, 특히 IL-1 β , IL-8, IL-17, MCP-1은 치료 8주 후 통계적으로 유의하게 감소되었다. 연구 기간 중 약제에 의한

이상반응은 보고되지 않았다.

본 연구를 통해 단기간의 1% 메틸프레드니솔론 점안제 치료가 유해한 이상반응 없이 임상지표를 호전시키고, 눈물내 염증성 싸이토카인 및 오스몰 농도를 감소시키는 것을 확인할 수 있었다. 현재 널리 사용되는 임상적인 지표에 더불어, 염증성 싸이토카인과 눈물내 오스몰 농도의 측정도 건성안 환자의 치료에서 객관적인 평가법으로써 사용될 수 있을 것이다.

핵심되는 말 : 건성안, 염증성 싸이토카인, 눈물내 오스몰 농도,
1% 메틸프레드니솔론