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ARTICLE

Evaluation of ocular surface disease in elderly patients with glaucoma: expression of matrix metalloproteinase-9 in tears

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Abstract

Purpose To evaluate the symptoms and signs of ocular surface disease (OSD) and tear-film matrix metalloproteinase-9 (MMP-9) overexpression using point-of-care testing (InflammaDry test) in patients with primary open-angle glaucoma (POAG).

Methods This prospective, case-control study included 67 patients diagnosed with POAG and 47 healthy control subjects. The OSD assessment included Schirmer-I test, the Oxford corneal stain scale, tear breakup time (TBUT), and the five-item dry eye questionnaire (*DEQ-5*). Measurement of extracellular MMP-9 level was performed using the InflammaDry test. The OSD parameters and MMP-9 expression levels were compared between the POAG group and the control group. Additional subgroup analysis in POAG group was performed according to number of topical glaucoma medications (Bottle 1, 2, or 3 medications).

Results There were significant differences between the control and POAG groups for all OSD parameters. MMP-9 overexpression was observed in 71.6% of POAG group, whereas only 31.9% of control group showed MMP-9 overexpression. The subgroup analysis revealed that *DEQ-5*, Oxford stain score, Schirmer-I, and MMP-9 overexpression demonstrated no significant difference among the three groups. Abnormal TBUT (≤5 s) was observed in 37.5%, 59.1%, and 76.2% for each subgroup according to number of bottles (1, 2, and 3), and strong MMP-9 overexpression were also detected in 25.0%, 40.9%, and 61.9%, respectively ($P = 0.032$, $P = 0.043$).

Conclusions The use of preservative-containing medications may affect the ocular surface in patients with POAG. Graded measurement of tear-film MMP-9 could provide more information on OSD and might be a more useful marker for inflammation than then conventional results obtained by using an MMP-9 kit.

Introduction

The goal of glaucoma treatment is to maintain the patient's visual function and quality of life (QoL) at a sustainable cost [1]. Since topical glaucoma medications are often used in treatment of glaucoma, it is essential for the patient to feel comfortable using antiglaucoma eye drops. However, a previous study showed that many patients with glaucoma who are on long-term topical medication have a high prevalence of signs and subjective symptoms of dry eye, which include irritation, ocular pain, photophobia, blurring, and visual fatigue [2]. Both the active components and the preservatives, such as benzalkonium chloride (BAK) and polyquaternium-1 (PQ), in topical medications may affect the ocular surface and induce squamous metaplasia of the conjunctival epithelium, and proinflammatory cytokines along with a decrease in the number of goblet cells [2, 3]. As a result, coexisting dry eye itself or intolerance to eye drops may reduce patient adherence to the treatment plan and consequently lead to disease progression [2, 3]. To maintain patient QoL, approaches to ocular surface disease (OSD) should not only consist of a simple diagnostic test for dry eye evaluation.

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Evaluation of ocular surface and inflammatory changes in patients with glaucoma is vital for maintaining QoL and visual function. However, there may be a discrepancy between conventional diagnostic test results and a patient's subjective symptoms. One reason for this difference is that Schirmer test is unrepeatable due to the reflex tear produced as a result of its irritating nature [4, 5]. Examination of tear breakup time (TBUT) may also be sometimes unreliable in these patients because use of topical anesthetic agents might destabilize the tear film and lead to an artificially accelerated TBUT [5, 6]. Moreover, neither Schirmer test nor TBUT provides direct information regarding ocular surface inflammation or elevated level of matrix metalloproteinase-9 (MMP-9) [7].

Evaluation of tear-film MMPs is essential in management of patients as dictated by the presence or absence of significant inflammation because recognition of inflammation may allow for a more targeted therapeutic management of care [8]. The level of MMP-9 is elevated in patients with severe OSD, Meibomian gland dysfunction, Sjogren's syndrome, and glaucoma [9].

Recently, an MMP-9 point-of-care device (InflammaDry, Rapid Pathogen Screening Inc., Sarasota, FL, USA) demonstrated good agreement for confirming suspected dry eye disease. Patients with glaucoma often exhibit progressive OSD, the incidence and severity of which are unfortunately underestimated [10]. In patients with long-term use of glaucoma medication or multiple medications, OSD may have negative effects on adherence/compliance and possible surgical outcome, thereby influencing prognosis of treatment [1, 11, 12]. Thus, glaucoma treatment could be enhanced by obtaining comprehensive knowledge of OSD including MMP-9 [13]. Clinical decision making considering the ocular surface is important; for example, separate agent therapy versus fixed combination therapy or choosing surgery over multiple medications in advanced disease, because the former may induce or exacerbate OSD and hence lead to poor adherence and glaucoma progression. However, there have been no studies regarding MMP-9 overexpression in such patients according to number of topical glaucoma medications.

The purpose of the current study was to evaluate the relationship of ocular surface and tear MMP-9 overexpression with point-of-care tests in patients using topical glaucoma medication.

Materials and methods

Subjects

This prospective case-control study was approved by the Institutional Review Board of Daegu Veterans Health Service Medical Center (IRB No. VHSMC 18016). All participants provided informed consent to participate. This study was performed in accordance with the tenets of the Declaration of Helsinki. The participants were enrolled and investigated between January 2018 and July 2018 at Daegu Veterans Health Service Medical Center in South Korea.

Prior to the study, patients with glaucoma underwent a complete ophthalmological examination, including refraction, Goldmann applanation tonometry, gonioscopy, stereoscopic disc photography, red-free retinal nerve fiber layer (RNFL) photography, standard automated perimetry (Humphrey Field Analyzer 740 instrument; Swedish Interactive Threshold Algorithm Standard; Carl Zeiss Meditec, Dublin, CA, USA), and peripapillary RNFL thickness measurement using the RTVue spectral domain OCT (Optovue, Fremont, CA, USA). Patients with primary open-angle glaucoma (POAG) who had used one or more topical antiglaucoma eye drops for at least 6 months were recruited for the study. We enrolled the age-matched control (volunteer) subjects from clinic prospectively. Healthy control subjects ($n = 50$) similar in age to the patients (±2 years) but without eye diseases were recruited [14]. The control group was defined as (1) those without a glaucomatous optic nerve head or RNFL defect, visual field defect, or other retinal disease, (2) visual acuity of 20/40 or better, (3) an open anterior chamber angle, (4) no history of IOP-lowering treatment, (5) no severe media opacities, (6) a refractive error between −6 and +4 diopters.

Exclusion criteria for both groups were as follows: (1) active inflammation or infection such as conjunctivitis, keratitis, and uveitis, (2) a recent history of ocular surgery <6 months prior to enrollment, (3) severe Meibomian gland dysfunction, (4) recent trauma, (5) contact lens use, or (6) allergy. In addition, patients using topical or systemic corticosteroids or cyclosporine, medications that are known to suppress MMP-9 expression, were excluded. The participants in this study underwent five-item dry eye questionnaire (*DEQ-5*), MMP-9 measurement, and ocular surface examinations sequentially.

In most glaucoma patients, prostaglandin analogs (PG) are prescribed as the first-line and first-choice treatment for IOP lowering. During the follow-up period, a glaucoma medication was added (1) if the IOP exceed 18 mmHg, in the case of concerning optic nerve or visual field changes given that the landmark Advanced Glaucoma Intervention Study [15] demonstrated that VF progression is delayed when IOP is consistently maintained below 18 mmHg, or (2) failure to reach the customized "Target IOP" [1, 16]. In general, followed PG, fixed combination drugs (timolol-dorzolamide, timolol-brinzolamide, or timolol-brimonidine) were added as the second-line drug to minimize exposure to preservatives and to lower IOP more effectively [1].

Subjective ocular symptom assessment using the DEQ-5

The *DEQ-5* was given to all participants to evaluate their dry eye-related symptoms [17]. The *DEQ-5* is a five-item, disease-specific QoL questionnaire used to quantify the impact of dry eye. It is the sum of scores for frequency and post meridiem intensity of dryness and discomfort plus the frequency of watery eyes, which are each scored by the patient. Recently, the Tear Film and Ocular Surface Society Dry Eye Workshop II Diagnostic Methodology Subcommittee recommended the *DEQ-5* or the ocular surface disease index (OSDI) to evaluate subjective patient symptoms as a diagnostic test [18]. The consensus view of the committee is to use the OSDI due to its strong establishment in the field or the *DEQ-5* due to its short length and discriminative ability. Many previous dry eye disease studies have used the *DEQ-5* to discriminate dry eye [17, 19–21]. Based on this, the present authors used the *DEQ-5*. A positive result was a *DEQ-5* score ≥ 6, and a strong positive (suspected Sjogren's syndrome) was a *DEQ-5* score ≥ 12 [17].

Measurement of MMP-9 using InflammaDry

InflammaDry is a 10-min, in-office immunoassay designed to detect elevated MMP-9 level (>40 ng/ml). The MMP-9 test was performed according to the manufacturer's instructions. No drops were placed in the patient's eye within 2 h of beginning the test. Briefly, the palpebral conjunctiva was gently dabbed eight to ten times in multiple locations, and the eyelid was released after every two to three dabs to allow the patient to blink. The absorbent tip was immersed in the buffer vial for 20 s and then laid flat on a horizontal plane for 10 min. The test was read and graded as follows; (1) negative and (2) weakly positive or broken (Grade 1), (3) positive (Grade 2), or (4) strongly positive (Grade 3, Fig. 1).

Ocular surface sign evaluation using TBUT, Oxford grading, and Schirmer test

At least 30 min after the MMP-9 point-of-care examination, a routine evaluation of the ocular surface was performed. TBUT was measured at the infero-temporal bulbar conjunctiva 2 min after instillation of fluorescein with a moistened fluorescein strip (Haag-Streit AG, Koniz, Switzerland). The interval between blinking and the appearance of the first break or a dry spot on the tear film was measured [5]. An abnormal TBUT was defined as follows: (1) TBUT ≤ 5 s (strict criteria) and (2) TBUT ≤ 10 s (lenient criteria). The Oxford grading scheme was used to evaluate ocular surface damage. Staining of the ocular surface upon slit-lamp examination was compared with a series of panels with staining as follows: grade 0 (absent), grade 1 (minimal), grade 2 (mild), grade 3 (moderate), grade 4 (marked), and grade 5 (severe) [22]. Schirmer-I test was performed without topical anesthetics. Schirmer strips were inserted into the inferior fornix and the junction of the lateral and middle thirds of the fornix, and then the patient was instructed to close her/his eyes. The strip was measured

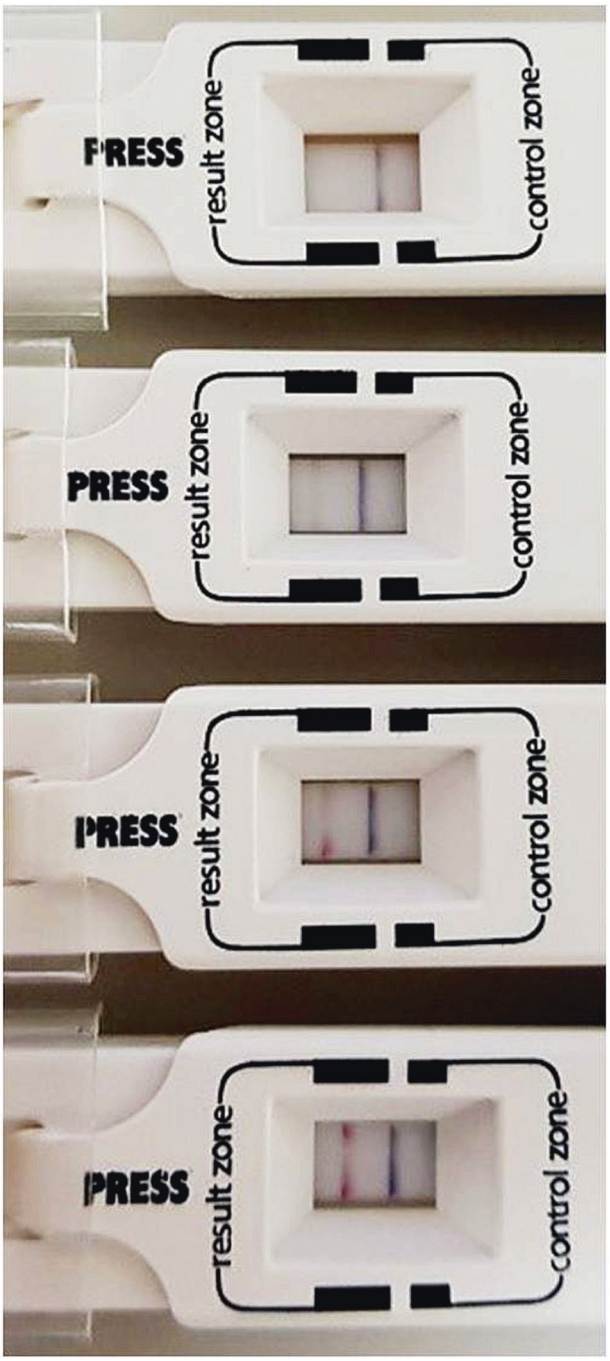

Fig. 1 Measurement of MMP-9 using InflammaDry (grading). 1, negative; 2, weakly positive; 3, positive; 4, strongly positive.

and recorded after 5 min. An abnormal Schirmer-I test was defined as (1) Schirmer-I test score ≤ 5 mm (strict criteria), and (2) Schirmer-I test score ≤ 10 mm (lenient criteria).

Statistical analysis

All data analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 18.0; SPSS Inc., Chicago, IL, USA). The sample size was estimated using MedCalc (version 15.8; MedCalc, Inc., Ostend, Belgium) on the basis of type I error of 5% and type II error of 10%. We assumed the proportion of dry eye disease in the control and glaucoma patients to be 30.3% and 61%, respectively, based on previous studies [10, 23]. The estimated sample sizes were 50 participants in the control group and 76 in the POAG group. Normality of distribution was tested with Kolmogorov–Smirnov test. Subgroup analysis in POAG patients was performed according to the number of topical glaucoma medications containing preservatives. Number of medications was defined as the number of antiglaucoma eye-drop "bottles" including preservative, not separate active molecules. Duration of disease was defined as period since diagnosis of glaucoma by medical history, and classified to longer-duration group (≥24 months) and shorter-duration group (<24 months). A comparison of the variables in the POAG and control groups was performed using an independent *t*-test, analysis of variance, and the Chi-square test. The level of statistical significance was set at $P < 0.05$.

Results

The study initially involved 125 eyes from 125 participants who met the inclusion criteria: 75 eyes in 75 POAG patients and 50 eyes in 50 age-matched volunteers without glaucoma medication as the controls. No individual declined participation in this study. Of the 75 POAG patients, 8 were excluded due to recent ocular surgery ($n = 3$), use of topical steroid or cyclosporine medications ($n = 4$), or inadequate results of MMP-9 kits ($n = 1$). Of the 50 control subjects, 3 were excluded due to use of topical steroids ($n = 3$). The final samples were 67 eyes in 67 POAG patients and 47 eyes of 47 control participants.

Comparison of OSD parameters

The study included 59 men and 8 women (mean age: 72.7 ± 4.3 years) in the POAG group and 43 men and 4 women (mean age: 73.7 ± 4.5 years) in the control group (Table 1). Age, sex ratio, ratio of diabetes, and hypertension were not significantly different between the POAG and control groups. The intraocular pressure (IOP) measured in the control group was significantly lower than that in the glaucoma group (14.2 ± 3.1 vs. 15.7 ± 3.2, $P = 0.013$). After a subjective ocular symptom comparison, the number of patients with *DEQ-5* score ≥ 6 in the POAG group was higher than that in the control group (62.7% vs. 40.4 %, $P = 0.019$). In regard to objective ocular symptoms, the ratios of TBUT score ≤ 5 s and ≤10 s in the POAG group were higher than those of the control group (56.7% vs. 27.7%, $P = 0.002$ and 82.1% vs. 65.9%, $P = 0.049$). The ratio of patients with Oxford stain score ≥ 1 in the glaucoma group was higher than that of that in the control group (34.3% vs. 2.1%, $P = 0.041$). In addition, the ratios of patients with Schirmer-I score ≤5 mm and ≤10 mm in the POAG group were higher than those in the control group

Table 1 Characteristics of the study participants.

Variables	Control participants $N = 47$	Glaucoma patients $N = 67$	*P* value*
Age, years	73.7 ± 4.5 (62–84)	72.7 ± 4.3 (63–86)	0.235
Male/female	43/4	59/8	0.557
Diabetic mellitus (%)	11 (23.4%)	19 (28.3%)	0.554
Systemic hypertension (%)	14 (29.8%)	22 (32.8%)	0.730
IOP, mmHg	14.2 ± 3.1 (range: 9–21)	15.7 ± 3.2 (range: 10–27)	0.013
DEQ-5			
≥6 *n* (%)	19 (40.4)	42 (62.7)	0.019
≥12 *n* (%)	12 (25.5)	27 (40.3)	0.102
TBUT			
≤10 s *n* (%)	31 (65.9)	55 (82.1)	0.049
≤5 s *n* (%)	13 (27.7)	38 (56.7)	0.002
Stain score			
≥1 *n* (%)	8 (2.13)	23 (34.3)	0.041
≥3 *n* (%)	2 (4.26)	3 (4.5)	0.955
Schirmer-I test score			
≤10 mm *n* (%)	25 (53.2)	49 (73.1)	0.028
≤5 mm *n* (%)	13 (27.7)	37 (55.2)	0.004
MMP-9			
Positive *n* (%)	15 (31.9)	48 (71.6)	<0.001
Strongly positive *n* (%)	6 (12.7)	28 (41.8)	0.001
Visual field (MD), dB	NA	−8.32 ± 6.13 (range, −0.48 to −27.0)	
Visual field (VFI), %	NA	80.95 ± 15.41 (range, 11–99)	

BCVA best-corrected visual acuity, *IOP* intraocular pressure, *MD* mean deviation, *VFI* visual field index.

**P* value obtained using the independent *t*-test for numeric data. For discrete data, a Chi-square test was used.

(55.2% vs. 27.7%, $P = 0.002$ and 73.1% vs. 53.2%, $P = 0.004$, $P = 0.028$, respectively). In particular, MMP-9 overexpression (≥Grade 1) was observed in 71.6% of POAG patients, whereas only 31.9% of controls were positive for MMP-9 overexpression ($P < 0.001$). In addition, the strongly positive values on the MMP-9 test in the glaucoma group were significantly higher than those in the control group (41.8% vs. 12.7%, $P = 0.001$).

Subgroup analysis of OSD parameters

When the OSD parameters were compared according to number of glaucoma medications (bottles) containing preservatives (0 as a control, and then 1, 2, and 3), *DEQ-5* score ≥ 6, TBUT score ≤ 5 s, Oxford stain score ≥ 1, Schirmer-I score ≤ 5 mm, and positive result (≥Grade 1) and a strongly positive result (Grade 3) on the MMP-9 test all had statistical significance (Table 2). A subgroup analysis within the POAG groups (medication 1, 2, and 3) revealed that the *DEQ-5*, Oxford stain score, Schirmer-I test, and MMP-9 score demonstrated no significant difference among the three groups (Table 3). However, abnormal TBUT score ≤ 5 s was observed in 37.5%, 59.1%, and 76.2% of participants, respectively ($P = 0.032$). Moreover, strongly positive results obtained using the MMP-9 kit were detected in 25.0%, 40.9%, and 61.9% of participants for each group, respectively ($P = 0.043$). Duration since diagnosis was 32.5 ± 20.6, 46.0 ± 29.2, 56.3 ± 30.2 months for each group, respectively ($P = 0.015$)

Regarding the effect of duration on MMP-9 positivity, the longer-duration group (≥24 months) and shorter-duration group (<24 months) were not significantly different (69.3% [9 positive cases of 13 patients] vs. 72.2% [39/54], $P = 0.860$). Interestingly, the longer-duration group demonstrated a higher proportion of strong MMP-9 overexpression than the shorter-duration group (48.2% [2/13] vs. 15.4% [26/54], $P = 0.032$). The relative risk for strong MMP-9 positivity was 5.017 times (95% CI, 1.033–25.256) greater in the longer-duration group.

Sub-analysis in a single-medication group

We performed additional sub-analysis in a single-medication group to investigate the effect of a particular active molecule and concentration and type of preservatives (BAK or PQ) on the MMP-9 results. Figure 2 summarizes the various ocular surface indices of the single-medication group. However, in most patients, PG analogs are prescribed as the first-line treatment for glaucoma. Thus, these results did not reach statistical significance for intra-class (between PG analogs) and inter-class differences (between PG and other classes of drugs [beta-blockers, alpha-agonists, or carbonic anhydrase inhibitors]) due to small sample size for each medication. Although there is no statistical significance, travoprost with PQ demonstrated relatively favorable result.

Table 2 Comparison of ocular surface disorder (OSD) parameters according to number of glaucoma medications containing preservatives.

Medication numbers	Control 0[a]	Glaucoma patients			*P* value*
		1	2	3	
	$N = 47$	$N = 24$	$N = 22$	$N = 21$	
DEQ-5					
≥6 *n* (%)	19 (40.4)	11 (45.8)	15 (68.2)	16 (76.2)	0.018
≥12 *n* (%)	12 (25.5)	7 (29.2)	9 (40.9)	11 (52.4)	0.147
TBUT					
≤10 s *n* (%)	31 (65.9)	17 (70.8)	19 (86.4)	19 (90.5)	0.088
≤5 s *n* (%)	13 (27.7)	9 (37.5)	13 (59.1)	16 (76.2)	0.001
Oxford stain score					
≥1 *n* (%)	8 (2.13)	5 (20.8)	8 (36.4)	10 (47.6)	0.040
≥3 *n* (%)	2 (4.26)	0 (0)	1 (4.5)	2 (9.5)	0.489
Schirmer-I test score					
≤10 mm *n* (%)	25 (53.2)	15 (62.5)	17 (77.3)	17 (80.9)	0.081
≤5 mm *n* (%)	13 (27.7)	10 (41.7)	12 (54.5)	15 (71.4)	0.006
MMP-9					
Positive *n* (%)	15 (31.9)	14 (58.3)	17 (77.3)	17 (80.9)	<0.001
Strongly positive *n* (%)	6 (12.7)	6 (25.0)	9 (40.9)	13 (61.9)	<0.001

Number of medications was defined as the number of antiglaucoma eye-drop "bottles" including preservative not separate active molecules.

[a]Category 0 is the control group and 1, 2, and 3 are the glaucoma patients.

*P value obtained using a Chi-square test.

Discussion

In our study, 62.7% of patients in the POAG group reported subjective symptoms (*DEQ-5* score ≥ 6), 73.1% of patients had a decrease in tear production (≤10 mm), 34.3% of patients demonstrated ocular surface staining, and 56.7% of patients showed a short TBUT (≤5 s). These results are quite similar to those of previous studies regarding the prevalence (59–64%) of OSD in glaucoma patients [10, 24]. These findings suggest a large proportion of patients with glaucoma to have signs and symptoms of OSD.

In particular, MMP-9 overexpression (≥Grade 1) was observed in 71.6% of POAG patients, whereas only 31.9%

Table 3 Subgroup analysis of ocular surface disorder (OSD) parameters according to number of glaucoma medications containing preservatives in the POAG group.

Medication numbers	1 $N = 24$	2 $N = 22$	3 $N = 21$	P value*
DEQ-5				
≥6 *n* (%)	11 (45.8)	15 (68.2)	16 (76.2)	0.089
≥12 *n* (%)	7 (29.2)	9 (40.9)	11 (52.4)	0.285
TBUT				
≤10 s *n* (%)	17 (70.8)	19 (86.4)	19 (90.5)	0.188
≤5 s *n* (%)	9 (37.5)	13 (59.1)	16 (76.2)	0.032
Oxford stain score				
≥1 *n* (%)	5 (20.8)	8 (36.4)	10 (47.6)	0.163
≥3 *n* (%)	0 (0)	1 (4.5)	2 (9.5)	0.305
Schirmer-I test score				
≤10 mm *n* (%)	15 (62.5)	17 (77.3)	17 (80.9)	0.329
≤5 mm *n* (%)	10 (41.7)	12 (54.5)	15 (71.4)	0.134
MMP-9				
Positive *n* (%)	14 (58.3)	17 (77.3)	17 (80.9)	0.189
Strongly positive *n* (%)	6 (25.0)	9 (40.9)	13 (61.9)	0.043
Approximate duration of disease by history (months)				
(Range)	32.5 ± 20.6 (6–96)	46.0 ± 29.2 (12–120)	56.3 ± 30.2 (12–120)	0.015

Number of medications was defined as the number of antiglaucoma eye-drop "bottles" including preservative not separate active molecules.

*P value obtained using a Chi-square test or analysis of variance.

of controls had a positive result on the MMP-9 test in our study. One previous study [25] reported that MMP-9 overexpression was related with use of BAK, with 46.7% of participants treated with BAK-containing medication showing overexpression but just 16.7% of participants in each of the untreated and preservative-free medication groups demonstrating overexpression. However, these researchers did not perform a subgroup analysis according to number of glaucoma medications being used. In this regard, our findings suggest that the number of glaucoma medications related to MMP-9 overexpression.

When comparing the OSD parameters according to the number of glaucoma medications containing preservatives, all parameters showed significant or borderline significant differences in our study. In contrast, a subgroup analysis in POAG patients showed only short TBUT score and strong MMP-9 positivity (Grade 3) showed statistically significant differences. Thus, graded measurement of tear-film MMP-9 could provide more information on OSD and might be a more useful marker for inflammation than conventional results from the MMP-9 kit.

Our results raise questions about the reason for MMP-9 overexpression in glaucoma patients who use topical medications and the clinical importance of these changes. We postulate that the reasons for MMP-9 overexpression in the present study are related to (1) inflammation, as the preservatives in glaucoma medication may impact the inflammatory response on the ocular surface, and (2) the possibility of coexistence of OSD and glaucoma itself.

MMP-9 has central importance in cleaving epithelial basement membrane components and tight junction proteins that maintain corneal barrier function [26]. MMP-9 is produced by stressed corneal epithelial cells and lacrimal glands and inflammatory cells [5, 27]. Active components and preservatives, such as BAK and PQ, in topical glaucoma medications may have detrimental effects on the ocular surface [3, 28, 29] and induce squamous metaplasia of the conjunctival epithelium, goblet cell dropout [2, 3], and inflammatory changes in subepithelial conjunctiva and corneal epithelium [28, 29]. As result, exposure to preservative might affect MMP-9 positivity through inflammatory reaction. Second, OSD itself was more prevalent in the glaucoma medication group than in participants who did not use medications. This is supported by higher OSDI score, greater corneal staining and a lower tear meniscus height in glaucoma patients compared with the control group [24].

Based on the goals of treatment, which include QoL and IOP control, management of coexisting OSD in glaucoma patients is very important. Batra et al. demonstrated that controlling OSD resulted not only in an improvement in OSD, but also in better IOP control [12]. Although InflammaDry is not designed or intended to monitor OSD after initiation of treatment, some researchers have suggested that a combination of clinical variables along with various biomarkers, such as MMP-9, may be the most reliable prognostic factor of a patient's response to therapy [30]. Thus, identification of inflammatory factors in symptomatic dry eye-affected patients with glaucoma may guide therapeutic recommendations, including use of artificial tears, anti-inflammatory therapy, or punctual plugs.

In control group, a significant portion of control patients (~20–40%) seems to have showed abnormal findings for TBUT, DEQ-5, Schirmer test, and MMP-9. In previous studies based on elderly Korean population [23], crude prevalence of dry eye disease in subjects aged 65 or over was 30.3% and age, sex, and region-adjusted prevalence was 33.2% [23]. These findings are similar to our results and reflect characteristics of elderly population.

In the current study, the approximate duration of disease by history was 32.5 months (1 medication), 46.0 months (2 medications), and 56.3 months (3 medications) in subgroup analysis according to number of bottles, respectively ($P = 0.015$). The longer-duration group (≥24 months) demonstrated a higher proportion of strong MMP-9 overexpression than shorter duration (48.2% vs. 15.4%, $P = 0.032$). Similarly, there are many previous studies [10, 11, 31] confirmed the tendency of worsening dry-eye symptom with increasing duration of glaucoma treatment,

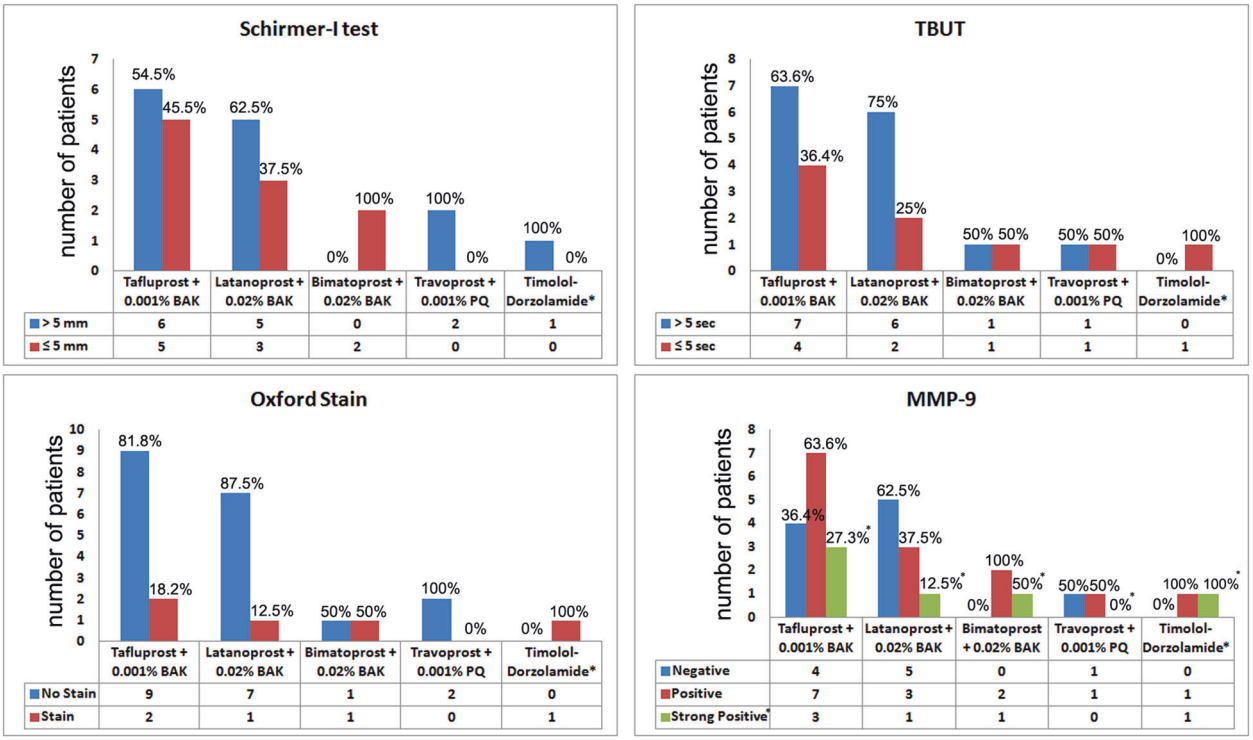

Fig. 2 Various ocular surface disease indices and MMP-9 overexpression of the single-medication group. *The category "Strong Positive" is derived from the "positive" category in measurement of MMP-9. Tafluprost 0.0015 + BAK 0.001%; Latanoprost 0.005 + BAK 0.02%; Bimatoprost 0.01 + 0.02% BAK; Travoprost 0.004 + 0.001% PQ; *Fixed combination drug (0.5% timolol + 2% dorzolamide) + 0.0075% BAK; BAK benzalkonium chloride, PQ polyquaternium-1, MMP-9 matrix metalloproteinase-9, Oxford Stain Oxford corneal stain scale, TBUT tear breakup time.

in terms of OSD symptom severity, lipid layer thickness, or other parameters. However, we could not evaluate the relationship among type of medication, duration of disease, and signs and symptoms of OSD exactly, because most patients were referred while already on topical medications [10] and accurate duration or cumulative effects could not be evaluated due to switching, add-on or cessation of medication. Thus, investigation of the effects of treatment duration is not main focus in this study. Despite these limitations, our results also suggest the possibilities that longer treatment duration is significantly associated with worsening of OSDs through inflammations.

The increasing number of medications prescribed may lead to further exposure to BAK or other preservatives [3] In this context, our results support that use of fewer glaucoma medications offers favorable results in terms of OSD. However, the ideal treatment for glaucoma should offer continuous effective IOP management along with good safety profiles [15]. Some medications had unique complications such as issues with compliance, tolerability, lid problem, and allergic reaction [10, 28]. Thus, selection of medications should be made after considering the glaucoma damage, life expectancy, risk factors, and status of patients [1, 3].

Our study had several limitations. First, the measurement of MMP-9 was based on the results of a point-of-care kit. Although our findings do not represent quantitative assays for ocular inflammation, the MMP-9 test is minimally invasive, is simple to conduct in clinical practice, and has been widely used in evaluation of OSD. Second, a large proportion of patients enrolled in this study were elderly male. These characteristics reflect demographics common in the veterans' affairs healthcare system. For this reason, there is a limit to extrapolating our findings to a general population. Third, we could not conclude the relationship between the duration of treatment and clinical sign and symptoms of OSD exactly. Further studies to clarify the effects of different preservatives or active components are needed.

In conclusion, use of preservative-containing medications may affect the ocular surface and subsequent inflammation in patients with POAG. In particular, graded measurement of tear-film MMP-9 could provide more information on OSD and might be a more useful marker for inflammation than conventional results from an MMP-9 kit.

Summary

What was known before

- The use of preservative-containing medications may affect the ocular surface in patients with POAG.
- Glaucoma patients treated with BAK-containing medication had higher tear MMP-9 levels and more rapid TBUT compared with those treated with preservative-free medication.

What this study adds

- Subgroup analysis in POAG patients according to number of topical glaucoma medications showed only short TBUT score and strong MMP-9 positivity (Grade 3) showed statistically significant differences.
- Graded measurement of tear-film MMP-9 could provide more information on OSD and might be a more useful marker for inflammation than conventional results obtained using an MMP-9 kit.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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