

# Diquafosol ophthalmic solution on ocular surface parameters in visual display terminal-associated dry eye disease

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## Abstract

• **AIM:** To evaluate 3% diquafosol ophthalmic solution on ocular surface parameters and the alterations of lipid and muco-aqueous layer in tear film of patients with visual display terminal (VDT)-associated dry eye disease (DED).

• **METHODS:** This study included patients with VDT-associated DED. It was a prospective single-arm interventional clinical trial. Patients were provided with 3% diquafosol ophthalmic solution for 3mo and

were followed up in 1, 2 and 3mo after treatment. Tear breakup time (TBUT), ocular surface staining score, and ocular surface disease index (OSDI) score were ocular surface characteristics. Lipid layer thickness (LLT), tear meniscus height (TMH), and mucin mRNA expression levels (MUC1, MUC4, MUC5AC, MUC16, and MUC20) were used to measure changes in the tear film. The LipiView interferometer was used to measure the partial blink rate (PBR).

• **RESULTS:** Sixty-eight eyes of 68 participants (54 females; mean age 25.12±4.10y; mean spherical equivalent -4.35±2.69 D) were enrolled. Compared with the pre-treatment, OSDI scores and TBUT improved significantly at all follow-up time points (all  $P<0.01$ ), and TMH increased significantly at 1 and 3mo ( $P<0.01$ ,  $P<0.001$ , respectively). Conjunctival lissamine green staining improved only at 2mo ( $P<0.05$ ), while corneal fluorescein staining showed no significant changes. Overall LLT remained unchanged, but the PBR<1 subgroup exhibited significant LLT elevation at 3mo ( $P<0.05$ ), unlike the PBR=1 subgroup. Conjunctival mRNA expression of MUC1, MUC5AC, MUC16, and MUC20 was significantly upregulated at 1 and 3mo (all  $P<0.01$ ), and MUC4 expression increased significantly only at 1mo ( $P<0.001$ ).

• **CONCLUSION:** In patients with VDT-associated DED, 3% diquafosol ophthalmic solution dramatically reduced symptoms and enhanced tear film stability by promoting ocular surface muco-aqueous secretion. Patients with better blinking habits (PBR<1) demonstrate greater LLT improvement than those with poorer habits.

• **KEYWORDS:** dry eye disease; visual display terminal; diquafosol; mucin

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## INTRODUCTION

Visual display terminals (VDTs) have grown to be an increasingly integral part of work and daily life worldwide because of the proliferation of the internet and electrical devices. The number of global internet users was reported to be 2.5 billion in 2013 and has since risen to approximately 5.56 billion in 2025<sup>[1]</sup>. With the extensive use of VDTs, side effects associated with prolonged exposure have gained increasing attention, including psychological stress, musculoskeletal disorder, asthenopia and dry eye disease (DED)<sup>[2]</sup>.

DED, which results from loss of tear film homeostasis, is one of the most common ocular conditions encountered in the ophthalmology outpatient department<sup>[3]</sup>. Its global prevalence has been estimated at 11.59%<sup>[4]</sup>, while among VDT users, the prevalence ranges widely from 26% to 70% according to a Meta-analysis<sup>[5]</sup>. This proportion rapidly increased during the COVID-19 pandemic<sup>[6]</sup>. A higher prevalence among female VDT users (76.5%) compared to males (60.2%) was reported in the Osaka study<sup>[7]</sup>.

The development of DED is aided by the evaporation of tear fluid and mucin loss in the ocular surface in VDT users, as well as the decreased blink rates and increased number of incomplete blinks<sup>[8-9]</sup>. VDT-associated DED not only impairs quality of life and work productivity but also poses a substantial economic burden on society<sup>[10]</sup>. Consequently, it has emerged as a significant public health issue, underscoring the need for effective management strategies.

Current preventive measures for VDT-associated DED primarily aim to stabilize the tear film<sup>[11]</sup>. Devices were employed to treat and prevent DED in VDT users, such as warming eye masks, glasses, and goggles<sup>[12-14]</sup>. Recent studies investigated that transcutaneous electric nerve stimulation<sup>[15]</sup> and acupuncture steam-warming eye mask<sup>[13]</sup> which were shown to alleviate some subjective DED symptoms in VDT users. However, the efficacy of these approaches is often limited and not well sustained.

Recently, 3% diquafosol ophthalmic solution, a P2Y2 receptor agonist, has been reported for treating DED in VDT users. Several reports indicate that diquafosol improves both symptoms and clinical signs of VDT-associated DED<sup>[16-17]</sup>. Experimental evidence shows that the P2Y2 receptor is expressed in the corneal epithelium, meibomian gland ductal epithelium, and lacrimal gland ductal epithelium in addition to the conjunctival epithelium containing goblet cells<sup>[18-20]</sup>. It is thus plausible that topical secretagogues like diquafosol may enhance both the lipid and muco-aqueous layers of the tear film in VDT-associated DED patients. Nevertheless, the long-term efficacy of such treatment warrants further investigation.

To the best of our knowledge, few clinical studies have comprehensively evaluated the effects of topical secretagogue treatment on each layer of the tear film in patients with VDT-associated DED. Therefore, the purpose of this trial was to determine the effectiveness of a topical secretagogue (3% diquafosol ophthalmic solution) in treating VDT-associated DED and to investigate its possible mechanisms.

## PARTICIPANTS AND METHODS

**Ethical Approval** This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Peking University Third Hospital Medical Science Research Ethics Committee (M2020402). This clinical trial was registered on Clinicaltrials.gov (NCT04668118). Participants signed a written informed consent.

**Participants** This study was a prospective single-arm intervention clinical trial, which included 68 VDT-associated DED patients who were admitted to the ophthalmology clinic of Peking University Third Hospital and finalized this trial from February 2021 to October 2021.

Inclusion criteria were as follows: age 18-40 years old; using VDT for at least 5d a week with more than 4h a day; meet the diagnostic criteria for DED according to the 2020 Chinese expert consensus on DED<sup>[21]</sup>: 1) Ocular Surface Disease Index (OSDI) score  $\geq 13$  and fluorescein tear breakup time (TBUT)  $\leq 5$ s; 2) OSDI score  $\geq 13$ ; along with  $5s < TBUT \leq 10s$  and positive corneal and conjunctival staining. The patients voluntarily participated in this study.

Exclusion criteria were as follows: active ocular surface or intraocular inflammation; history of ocular allergy; experience of eye trauma or eye surgery; wearing contact lenses within the past three months; meibomian gland dysfunction or obvious inflammation of the eyelid margin; suffering from eye diseases that may affect tear production; pregnant or lactating women; poorly controlled systemic diseases such as diabetes, hypertension, *etc.*; patients with systemic or topical use of antibiotics or medications that affect the production of tears (except artificial tears); patients who have previously used diquafosol sodium eye drops or those who cannot tolerate and refuse to use them; patients with a history of anxiety and depression.

**Methods** The participants who were eligible for participation were provided with 3% diquafosol sodium eye drops (Diquas<sup>®</sup>, Santen Pharmaceutical, Osaka, Japan). They were instructed to drop their eyes six times each day for 3mo, and followed up at 1, 2, and 3mo after treatment. The alterations of ocular surface characteristics on the worse eye before and after treatment were analyzed and evaluated. Table 1 contains the assessment indicators for baseline and each time point following treatment.

**OSDI score** A total of 12 questions including ocular discomfort symptoms, visual function and the impact of

**Table 1 Assessment indicators for baseline and each time point after treatment**

Indicators	Baseline	1mo	2mo	3mo
OSDI score	Yes	Yes	Yes	Yes
TBUT	Yes	Yes	Yes	Yes
CFS score	Yes	Yes	Yes	Yes
LGS score	Yes	Yes	Yes	Yes
LLT	Yes	Yes	-	Yes
TMH	Yes	Yes	-	Yes
mRNA level of mucins	Yes	Yes	-	Yes
PBR	Yes	Yes	-	Yes

OSDI: Ocular Surface Disease Index; TBUT: Fluorescein tear breakup time; CFS: Corneal fluorescein staining; LGS: Lissamine green staining; LLT: Lipid layer thickness; TMH: Tear meniscus height; PBR: Partial blinking rate.

environmental factors on the eyes were scored according to the duration of each symptom. The higher the OSDI score, the more serious the ocular symptoms.

**Tear breakup time** The fluorescein sodium test strips and cobalt blue light were used to determine the time from the last blink to the point of tear film ruptured. The average value after three repetition was taken as TBUT.

**Corneal fluorescein staining score** Following the evaluation of the TBUT, the score of corneal fluorescein staining (CFS) was evaluated based on the National Eye Institute grading system.

**Conjunctival lissamine green staining score** The lissamine green staining (LGS) test strip was used, and the staining was observed with white light. Conjunctiva was divided into two quadrants, including temporal and nasal conjunctiva. The Oxford scale was also used to rate each quadrant

**Lipid layer thickness** LipiView interferometer (TearScience Inc, Morrisville, NC, USA) was adopted to evaluate lipid layer thickness (LLT). The interferometry color unit (ICU) of the tear film was assessed as an indicator of LLT. One ICU approximately reflects 1 nm of the LLT.

**Tear meniscus height** The lower tear meniscus height (TMH) was measured under infrared light without stimulating reflex tearing using ocular surface comprehensive analyzer (OCULUS Keratograph 5M, OCULUS, Germany). The average value of three measurements was recorded.

**Relative mRNA level of mucins** After instilling a drop of topical anaesthetic (0.4% oxybuprocaine hydrochloride, Benoxil, Santen, Japan), the conjunctival impression cytology of each participant was collected using sterilized nitrocellulose membrane and stored in 0.1%  $\beta$ -mercaptoethanol (Sigma-Aldrich) at -80°C fridge. Then the RNA extraction and reverse transcription were conducted following the manufacturer's instructions. The cDNA obtained above was used as a template to detect the expression of target mucins genes (*MUC1*, *MUC4*, *MUC5AC*, *MUC16*, and *MUC20*) with 7500 real-time

polymerase chain reaction (PCR) system (Applied Biosystems, Carlsbad, CA, USA). The relative mRNA expression of the target gene was calculated using the  $\Delta$ CT method.

**Partial blinking rate** Partial blinking rate (PBR) was measured by the LipiView interferometer (TearScience Inc, Morrisville, NC, USA). The term "normal blinking" refers to blinking that makes complete contact between the upper and lower lids. While partial blinking means the upper lid does not fully contact with lower lid, which was reported in the DED patients<sup>[22-23]</sup> and intensive users of VDTs<sup>[11,24]</sup>. The partial blinking and total blinking events were detected within 20s, and the PBR was calculated as the rate between the two.

All participants were examined by the same experienced examiner before and after undergoing 1, 2, and 3mo of treatment. The experiments for testing mRNA levels of mucins were conducted by two qualified technicians. The examiners and technicians were masked to the time point of each participant.

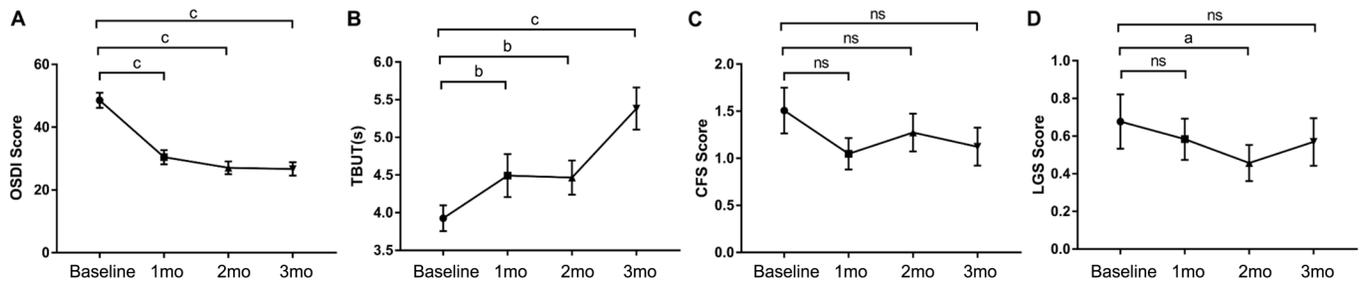
**Sample Size Calculation** The primary outcome measure of this study was TBUT. According to the relevant authoritative literature<sup>[25]</sup>, it was estimated that the average improvement of TBUT after the treatment of VDT-associated DED with 3% diquafosol sodium eye drops is 1.5, and the standard deviation is 4. According to the paired *t* test, the sample size was calculated as  $\alpha=0.05$ ,  $1-\beta=0.8$ , and the calculated sample size was 58 cases. Considering the possibility of 15% drop-off rate, at least 68 participants needed to be included.

**Statistical Analysis** The SPSS software version 22.0 and GraphPad Prism version 7.0 were applied for statistical analysis in this study. Comparisons of the data between each time point and baseline are conducted using the paired *t* test if the data is of the normal distribution, or otherwise the Wilcoxon signed rank test with the Bonferroni correction.  $P<0.05$  is considered to indicate a statistically significant difference.

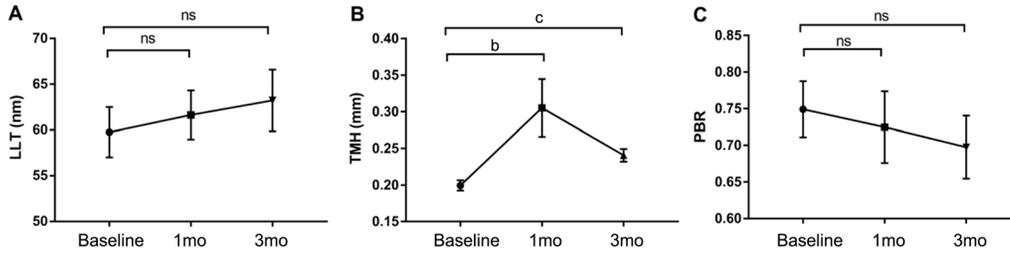
## RESULTS

A total of 72 participants were recruited in this study, of which four dropped out of the trial. One participant dropped out because of seasonal conjunctivitis, one dropped out because of a change of workplace, and two dropped out because of lost to follow-up. Therefore, 68 eyes of 68 participants were included for analysis, including 14 males and 54 females. The average age was  $25.12\pm 4.10$ y, and the average spherical equivalent was  $-4.35\pm 2.69$  D (Table 2).

**Changes of DED Characteristics** Compared to baseline, the OSDI score and TBUT at 1, 2, and 3mo after treatment were significantly improved (all  $P<0.01$ ; Figure 1A-1B). The OSDI scores showed a progressive decline throughout the 3-month treatment period. TBUT demonstrated significant improvement at 1-month follow-up, stabilized during the second month, then



**Figure 1** The changes of DED characteristics after treatment of diquafosol ophthalmic solution in VDT-associated DED patients OSDI: Ocular Surface Disease Index; TBUT: Fluorescein tear breakup time; CFS: Corneal fluorescein staining; LGS: Lissamine green staining; VDT: Visual display terminals; DED: Dry eye disease. <sup>a</sup> $P<0.05$ , <sup>b</sup> $P<0.01$ , <sup>c</sup> $P<0.001$ , ns: No significance.



**Figure 2** The changes of LLT, TMH, and PBR after treatment of diquafosol ophthalmic solution in VDT-associated DED patients LLT: Lipid layer thickness; TMH: Tear meniscus height; PBR: Partial blinking rate; VDT: Visual display terminals; DED: Dry eye disease. <sup>b</sup> $P<0.01$ , <sup>c</sup> $P<0.001$ , ns: No significance.

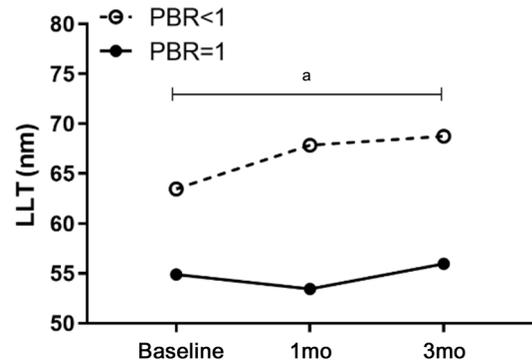
**Table 2** Demographics and baseline ocular surface parameters of the participants with VDT-associated DED

Demographics	Mean±SD
Female/male	54/14
Age, y	25.12±4.10
Spherical equivalent, D	-4.35±2.69
OSDI score	48.58±19.79
TBUT, s	3.93±1.41
CFS score	1.51±1.95
LGS score	0.68±1.16
LLT, nm	59.76±20.98
TMH, mm	0.19±0.06
PBR	0.75±0.32

OSDI: Ocular Surface Disease Index; TBUT: Fluorescein tear breakup time; CFS: Corneal fluorescein staining; LGS: Lissamine green staining; LLT: Lipid layer thickness; TMH: Tear meniscus height; PBR: Partial blinking rate; VDT: Visual display terminals; DED: Dry eye disease; SD: Standard deviation.

showed further progressive increase by the third month. There were no significant differences in CFS score at each time point after treatment (all  $P>0.05$ ; Figure 1C). LGS score only at 2mo after treatment was significantly different from that before treatment ( $P=0.024$ ; Figure 1D).

**Changes of LLT, TMH, and PBR** There were no significant differences in LLT at 1 and 3mo compared with baseline ( $P=0.429$ ,  $P=0.142$ ; Figure 2). TMH at 1 and 3mo after treatment was higher than that before treatment, and the differences were significant ( $P=0.002$ ,  $P<0.001$ ). PBR in VDT-associated DED patients showed no significant differences

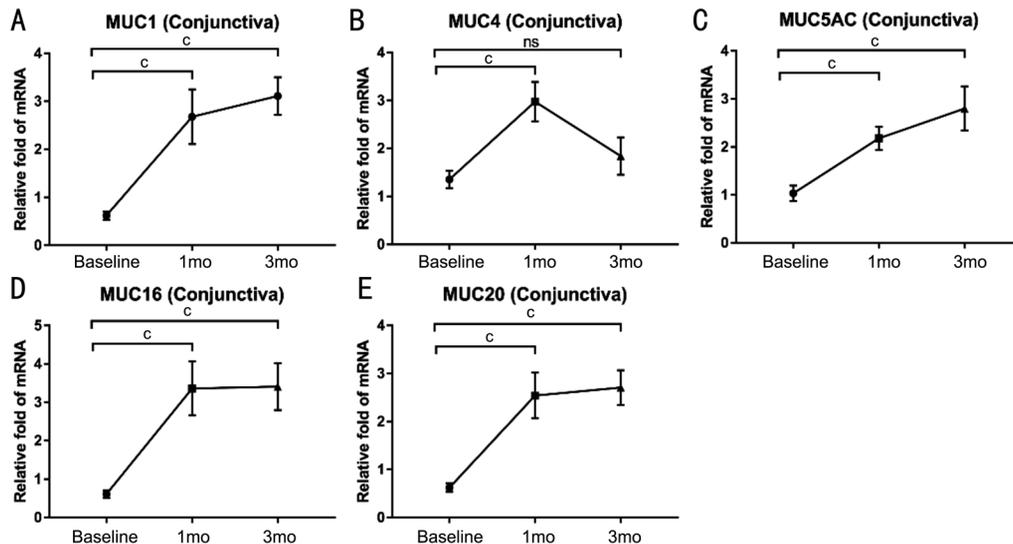


**Figure 3** The changes and comparisons of LLT in the sub-groups of VDT-associated DED patients After 3mo of treatment, the LLT in the PBR<1 group was significantly improved. LLT: Lipid layer thickness; PBR: Partial blinking rate; VDT: Visual display terminals; DED: Dry eye disease. <sup>a</sup> $P<0.05$ .

at each time point after treatment compared with baseline ( $P=0.097$ ,  $P=0.202$ ).

To further explore the changes in LLT in patients with VDT-associated DED with different habits after treatment, 68 subjects were divided into PBR=1 group ( $n=35$ ) and PBR<1 group ( $n=33$ ) for analysis. As shown in Figure 3, PBR<1 group showed a significant increase in LLT after 3mo of treatment ( $P=0.042$  compared with baseline), whereas PBR=1 group showed no significant change in LLT at each time point after treatment (all  $P>0.05$  compared with baseline).

**Changes of mRNA Expression of Mucins** The mRNA expressions of mucins in conjunctiva before and after treatment were evaluated and showed in Figure 4. The results of real-time PCR showed that the relative mRNA expressions



**Figure 4** The changes of mRNA expression of mucins after treatment of diquafosol ophthalmic solution in VDT-associated DED patients. MUC: Mucin; VDT: Visual display terminals; DED: Dry eye disease. <sup>c</sup> $P < 0.001$ , ns: No significance.

of *MUC1*, *MUC5AC*, *MUC16*, and *MUC20* at 1 and 3mo were significantly higher than those before treatment (all  $P < 0.001$ ). However, the relative mRNA expression of *MUC4* only increased significantly at 1mo after treatment ( $P < 0.001$ ), and there was no significant difference between 3mo after treatment and before treatment ( $P = 0.684$ ).

#### DISCUSSION

DED is a multifactorial ocular surface disease characterized by a loss of tear film homeostasis. The lipid layer and the mucoaqueous layer work together to maintain tear film stability, which guards the ocular surface epithelium against desiccation. Diquafosol, a dinucleotide derivative and purinergic P2Y2 receptor agonist, has demonstrated efficacy in various forms of DED, including post-cataract surgery DED<sup>[26]</sup>, post-small incision lenticule extraction DED<sup>[27]</sup>, and diabetes-associated DED<sup>[28]</sup>. While the effect of diquafosol on VDT-associated DED has been explored in a limited number of studies<sup>[16-17]</sup>. But these investigations have not thoroughly examined the effects of diquafosol on each layer of the tear film in patients with VDT-associated DED. This study may provide more solid evidences and individualized strategies for VDT-associated DED treatment. The pathophysiology of VDT-associated DED is directly related to tear film instability. Our previous investigation revealed that a deficiency in membrane-associated mucins within the conjunctival epithelium may contribute to tear film instability in VDT users<sup>[8]</sup>. Prolonged VDT use reduces blink rate and increases the frequency of incomplete blinks, promoting tear film instability, tear evaporation rates and hyperosmolarity. Hyperosmolarity further can amplify inflammatory mediators that damage the ocular surface and goblet cells. The subsequent loss of goblet cells impairs mucin secretion, further destabilizing the tear film and perpetuating a vicious cycle<sup>[5,11]</sup>.

In this study, instillation of 3% diquafosol ophthalmic solution significantly increased TBUT and reduced OSDI scores at 1, 2, and 3mo, demonstrating the ongoing efficacy of topical secretagogue treatment on the symptoms and signs of VDT-associated DED. These results were consistent with the research by Shimazaki *et al*<sup>[16]</sup>, who observed significant improvements in subjective symptoms and TBUT in office workers using computers for more than four hours daily after two and four weeks of diquafosol treatment. TBUT, a key indicator of tear film stability, is consistently enhanced by diquafosol, supporting its recommendation for short TBUT-type DED<sup>[29-30]</sup>.

However, ocular surface staining scores did not improve significantly after three months of treatment, suggesting that the effect of diquafosol on surface damage in VDT-associated DED may be limited. This observation is consistent with the findings of Wang *et al*<sup>[31]</sup>, who reported no statistically significant reduction in CFS after three months of diquafosol treatment following femtosecond laser-assisted laser *in situ* keratomileusis (FS-LASIK). The mild ocular surface staining observed in subjects with VDT-associated DED and post-refractive surgery DED may partly explain these results.

The tear film lipid layer, secreted by meibomian glands and spread during blinking, reduces evaporation and stabilizes the tear film. Proper blinking is essential for maintaining the lipid layer and stabilizing the tear film. Although prolonged screen time alters blinking behavior and exacerbates DED symptoms<sup>[24]</sup>, no significant changes in LLT or PBR were observed after three months of diquafosol treatment. According to a report by Fukuoka and Arita<sup>[32]</sup>, LLT in DED patients was considerably higher following topical instillation of diquafosol for 15, 30, and 60min. Collectively, these findings suggest that diquafosol may induce short-term lipid layer improvement but

lacks significant long-term effects on LLT improvement.

Subgroup analysis based on blinking habits revealed intriguing results. Patients were divided into two groups: PBR=1 group and PBR<1 group, taking partial blinking's impact on the lipid layer into consideration. After three months, LLT improved significantly only in the PBR<1 group. Jeon *et al*<sup>[22]</sup> reported PBR and meibomian gland dropout rate were positively associated. Besides, the meibomian gland was discovered to have P2Y2 receptor expression<sup>[18]</sup>. Diquafosol may directly activate P2Y2 receptors on the meibomian gland and leads to an increase in the meibomian glands secretions. The higher PBR, the higher meibomian gland dropout rate, and the lower P2Y2 receptor expression. Therefore, it is reasonable to assume that patients with normal blinking (*i.e.*, no partial blinking) may derive greater therapeutic benefit from diquafosol in VDT-associated DED, particularly regarding lipid layer improvement.

The aqueous layer is produced by the lacrimal glands and aims to lubricate the exposed ocular epithelium and to provide some nutrients, antimicrobial proteins and appropriate osmolarity. TMH is linearly proportional to the lacrimal secretory rate. The current study's finding of a significant improvement in TMH following treatment with 3% diquafosol ophthalmic solution supports the drug's efficacy in enhancing tear production, which is consistent with recent clinical evidence<sup>[31,33]</sup>. Diquafosol acts on P2Y2 receptors, which are found on the lacrimal gland. It increases the intracellular calcium and thereby helps in increasing the tear secretion<sup>[34]</sup>. On the other hand, despite receiving diquafosol treatment, neither Park *et al*<sup>[35]</sup> nor Mun *et al*<sup>[25]</sup> observed any appreciable improvement in Schirmer value. This may be explained by the possibility that various evaluation techniques were used across the different researches, which also indicating different methods may quantify different types of tears, such as basal, reflex, emotional, and closed-eye tears.

Except for fluid, diquafosol has been reported to stimulate the expression of secreted mucin and membrane-associated mucin on the conjunctiva goblet cells, conjunctival epithelial cells, and corneal epithelium<sup>[36-38]</sup>. Membrane-associated mucins on the ocular surface (MUC1, MUC4, MUC16 and MUC20) forms a kind of glycocalyx to prevent the eye from desiccation and bacterial adhesion<sup>[39]</sup>. MUC5AC is the most important secreted mucin on the ocular surface which help to remove pathogens and debris, and provide lubrication of the ocular surface for smooth blinking and good vision<sup>[40]</sup>. A few clinical studies testified decreased tear mucins in VDT users. According to Uchino *et al*<sup>[41]</sup>, MUC5AC concentrations in tears were low in DED patients who used VDTs and in long-term VDT users. Our previous study showed decreased MUC1, MUC16, and MUC20 expressions in the VDT users

with DED compared with that without DED<sup>[8]</sup>. To the best of our knowledge, no studies have been conducted to examine the changes in ocular surface mucin expression profiles after diquafosol therapy in VDT-associated DED patients. In the present study, topical diquafosol administration significantly upregulated mRNA expression levels of *MUC1*, *MUC16*, *MUC20*, and *MUC5AC* at both 1-month and 3-month follow-up intervals. In contrast, *MUC4* expression demonstrated only transient elevation at 1mo, with no statistically significant difference observed at 3mo compared to baseline levels.

Previous basic studies reported that diquafosol was effective for increasing both secreted mucin and membrane-associated mucin<sup>[36-37,42]</sup>. A recent study showed that in povidone iodine-induced dry eye model, increased mucins and conjunctival goblet cells density were observed in diquafosol group<sup>[38]</sup>. As for the clinical study, Shigeyasu *et al*<sup>[43]</sup> found diquafosol application significantly increased sialic acid, a marker for monitoring mucins in biological samples, suggesting that diquafosol stimulated the secretion of mucins from ocular tissues, but there was no direct detection for mucin levels. Notably, MUC4 exhibited a distinct expression pattern compared to other mucins, demonstrating limited improvement after the 1-month follow-up. It is currently unknown why the MUC4 level decreased one to three months after treatment. Intriguingly, we postulate that MUC4 expression may correlate with TMH alterations, given their parallel trends. However, this observed association currently lacks substantive support in published literature. The observed variation in MUC4 results may represent a unique, transient alteration, whose biological and clinical significance warrants further investigation. Future studies can increase the follow-up points of MUC4 levels and pay attention to the relationship between MUC4 and tear secretion volume. Our study provided the first confirmation of the elevated mRNA expressions of secreted mucins (MUC5AC) and membrane-associated mucins (MUC1, MUC16 and MUC20) on the human conjunctiva following diquafosol treatment in the VDT-associated DED patients.

There were two major limitations for this research. The primary methodological limitation of this study lies in its single-arm design. According to the study's objectives, this investigation concentrated on the effects of diquafosol on lipid and muco-aqueous layers of tear film following diquafosol treatment as a key finding. Next step, based on this study, is to prepare for a following study that will include a control group and different kinds of eye blink habits of VDT-associated DED patients to further explore significant markers. Although we observed significant improvements in multiple parameters, the lack of a parallel control group prevents us from fully excluding potential influences of placebo effects or natural disease fluctuations on the outcomes. Another drawback was

the fact that we only obtained conjunctival impression cytology samples; as a result, we measured mucin mRNA expression levels rather than protein levels in tear film. Further research is required to validate the consistency of increased mucin levels in tear samples and mRNA expression levels in conjunctiva samples following diquafosol treatment.

In conclusion, 3% diquafosol ophthalmic solution effectively improves dry eye symptoms and tear film stability in VDT-associated DED by enhancing aqueous and mucin secretion. The differential improvement in LLT among patients with varying blink efficiencies highlights the importance of considering blinking patterns in management strategies. Further large-scale studies, preferably multicenter randomized controlled trials, are warranted to validate these findings.

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**Data Availability Statement:** The dataset and materials generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Conflicts of Interest:** Yang TT, None; Duan HY, None; Zhou YF, None; Ma BK, None; Zhao L, None; Lan QQ, None; Chen JW, None; Wang XY, None; Yoon KC, None; Qi H, None.

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