• Clinical Research •

# Changes of tear film lipid layer thickness by 3% diquafosol ophthalmic solutions in patients with dry eye syndrome

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## Abstract

**• AIM: To evaluate the quantitatively changes in lipid layer thickness (LLT) when 3% diquafosol eye drop is used for dry eye patients using the tear film interferometer.**

**• METHODS: A total 124 participants (32 males, 92 females; mean age, 28.9y) diagnosed with dry eye disease (DED) received topical instillation of 4 ophthalmic solutions in one eye: diquafosol, normal saline, 0.1% sodium hyaluronate and 0.3% gatifloxacin, in a masked manner. LLT was measured using an interferometer at baseline and 20min after the instillation of each ophthalmic solutions.**

**• RESULTS: Changes of LLT after instillation (nm, mean± standard error) were as follows: 12.6±2.0 for diquafosol (*P*<0.001), 1.2±2.2 for normal saline (*P*=0.301), 1.5±2.0 for hyaluronate (*P*=0.495), and 0.5±3.2 for gatifloxacin (*P*=0.884).**

**• CONCLUSION: Topical instillation of diquafosol increases tear film LLT in DED patients. Diquafosol 3% eye drop might be effective treatment option of evaporative DED with meibomian gland dysfunction.**

**• KEYWORDS:** diquafosol ophthalmic solutions; dry eye syndrome; tear film lipid layer thickness

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## INTRODUCTION

Dry eye disease (DED) is one of the most difficult disease entities challenged by ophthalmologists. Globally, the incidence of DED varies from 5% to 50%, especially in Asia, with an incidence of over 30%[1]. DED is occurred by various causes, characterized by the numerous ocular symptoms including ocular irritation and visual disturbance, and which is accompanied by tear film instability, increased tear osmolarity, and ocular surface inflammation[2-3].

Recently topical 3% diquafosol eye drop can be one treatment option for an aqueous-deficient DED. Treatment using diquafosol eye drop for 4-6mo improved significantly both subjective symptoms and objective dry eye diagnostic parameters for aqueous-deficient type dry eye[4]. The long-term treatment of diquafosol reduced ocular higher-order aberrations, as well as corneal epithelial healing and improved tear film stability[5].

Diquafosol tetrasodium is an agonist of $P2Y_2$ purinergic receptor. It stimulates $P2Y_2$ receptors by activating fluid pump mechanism of lacrimal glands, causing tear component secretion[6-8]. $P2Y_2$ agonists are effective mucin secretagogues and stimulate the mucin secretion from conjunctival goblet cells as well. The capability of the $P2Y_2$ receptor activation to increase tear lipid component is not yet firmly proved, but past animal studies showed the existence of $P2Y_2$ receptor in the meibomian gland[9-10].

Tear film interferometer is a diagnostic device which evaluates tear film lipid layer thickness (LLT) by measuring ocular surface interference[11-14]. In the preceding studies[14-15], LLT was evaluated from grade 1 to grade 5 according to the patterns of color appearing on tear film interference. Recently, in order to gain the quantitative measurement of LLT, the LipiView (TearScience Inc, Morrisville, NC, USA) is adopted.

This study was designed to evaluate the changes of tear film LLT quantitatively after using 3% diquafosol ophthalmic solution and control ophthalmic solutions by tear film interferometer (Lipiview) in DED patients.

## SUBJECTS AND METHODS

**Ethical Approval** This prospective study was carried out

at the Department of Ophthalmology, Kim's Eye Hospital, Konyang University. The study was approved by the Institutional Review Board (IRB number: A-2015-004) at Kim's Eye Hospital, Seoul, Korea. All procedures of study followed the guidelines of the Declaration of Helsinki. Subjects were collected at the cornea clinic of Kim's Eye Hospital. To publish this study, informed consent forms were filled out by the participants themselves.

Each subject received following complete ophthalmic examinations: intraocular pressure with a applanation tonometer; best-corrected visual acuity (BCVA) and refractive error evaluated with autorefractor keratometer (TX-20P, Canon, Tokyo, Japan); anterior segment (upper and lower eyelid, conjunctiva, cornea, *etc*.) screening by slit-lamp microscopy. Dry eye screening examination was adopted to all subjects, including ocular symptoms by questioning, tear break-up time (TBUT) and ocular surface staining score by fluorescein dye.

The ocular surface was stained by sterile fluorescein strip with drop of sterile saline into the conjunctival sac. TBUT was recorded by measuring interval time between the last complete blinking and the initial break up of the stained ocular surface. Ocular surface staining score was graded from 0 to 3 onto the upper, middle, and lower third of the cornea. The score was given according to the aspects of stained ocular surface: 0, absent ; 1, minimal scattered stained dots; 2, moderate stained spotty; 3, marked diffuse stained area.

The Ocular Surface Disease Index (OSDI) by Allergan Inc, Irvine, CA, USA) is one of the most frequently used questionnaires for evaluation of DED. This includes 12 questions which measure the frequency of symptoms over the recent week, and the scores range from 0 to 100. According to the result, the patient's symptoms were classified as normal (from 0 to 12), mild (from 13 to 22), moderate (from 23 to 32), or severe (from 33 to 100) DED.

Meibomian gland expressibility was scored by using digital compression to the central one third area of the lower eyelid. The meibomian gland expressibility was classified into 0-3 score: 0 (clear meibum); 1 (cloudy meibum by mild compression); 2 (cloudy meibum by moderate compression); and 3 (no expressed meibum or toothpaste-like meibum by heavy compression)[16]. Higher meibomian gland expressibility means a more obstructive meibum secretion.

LLT was evaluated using the Lipiview interferometer by the principle of white light interferometry. The interferometry color units (ICU) of the tear film which is an indicator of LLT is assessed according to the mean interference color pattern through specular reflection[17]. Subjects who their the chin and brow were attached leaning forward against each stand were seated in front of the Lipiview interferometer. The test was performed with visual fixation of participants on the internal light-emitting diodes target. Experienced tester performed camera adjustment until the central pupil position and the reflected tear film image was in the targeting square area. During capturing images for 20s, tester allowed participants blink naturally. Meiboscore was also measured using meibograph of Lipiview interferometer: 0 (no meibomian gland loss); 1 (gland loss appeared less than one third of the total area); 2 (gland loss appeared between one third and two thirds); 3 (gland loss appeared more than two thirds).

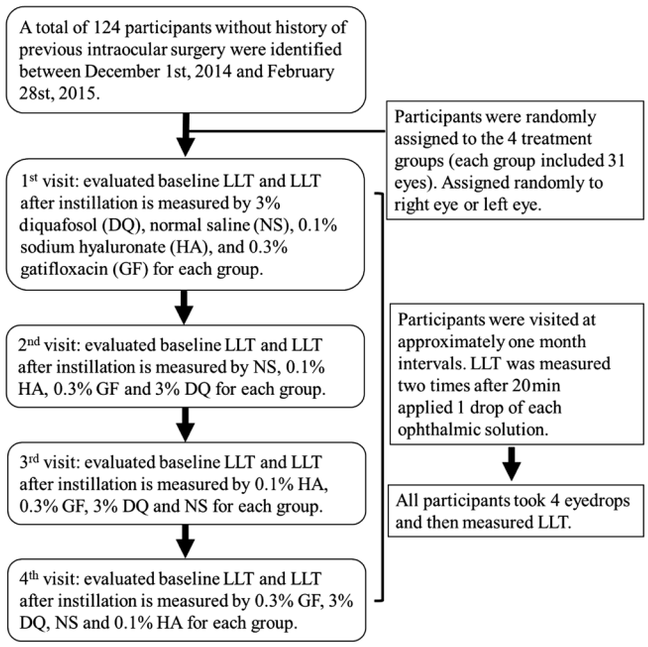


**Figure 1 Flow chart of this study.**

A total of 124 eyes of 124 participants were categorized randomly to the 4 treatment groups. All subjects received four tests. One eye (left or right eye) was randomly selected and to treated with four ophthalmic solutions (3% diquafosol eye drop, isotonic normal saline, 0.1% sodium hyaluronate, 0.3% gatifloxacin). Investigators were masked that treatment option of subjects remained unidentified status during the study. All subjects used a single topical eye drop in randomized selected eye. Participants were scheduled for a total of four visits and visited at approximately 1mo intervals. The LLT was measured after instillation of diquafosol eye drop, followed by normal saline, hyaluronate and gatifloxacin (Figure 1). LLT was measured two times after 20min of subjects applied 1 drop of each ophthalmic solution.

Inclusion criteria for screening DED were BCVA>20/30, TBUT<5s, corneal fluorescein staining score >1 by Oxford schema.

Exclusion criteria included allergic conjunctivitis and anterior segment diseases, previous consecutive topical eye drop user, contact lens wearer, those who has a history of ocular surgery recent 3mo and systemic diseases that would correlated with tear film dysfunction.

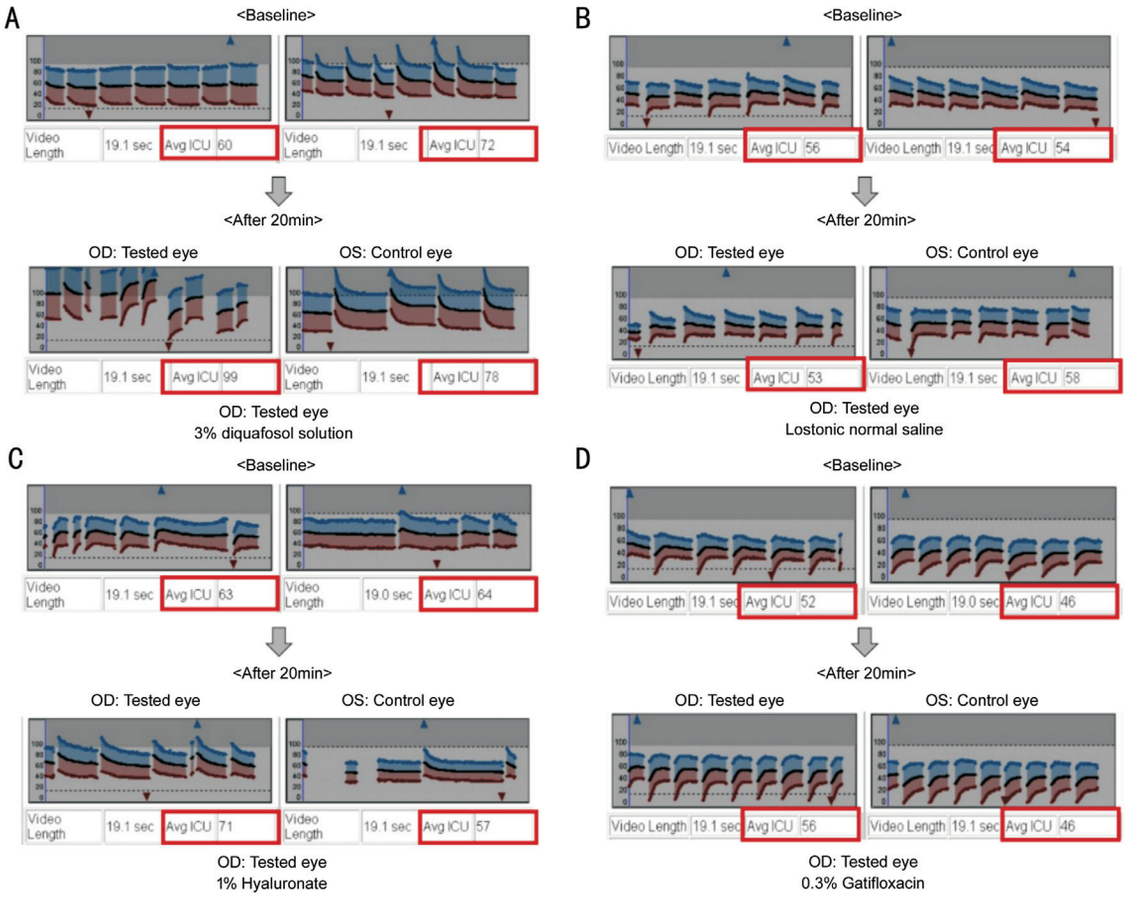


**Figure 2 Cases of LipiView interferometer for 4 ophthalmic solutions** A: A 34-year-old female with 3% diquafosol eye drop; B: A 36-year-old male with isotonic normal saline; C: A 27-year-old female with 0.1% hyaluronate: D: A 26-year-old female with 0.3% gatifloxacin.

**Statistical Analysis** Paired *t*-test in SPSS version 18.0 (SPSS Inc, Chicago, Illinois, USA) was used for statistical analyses. If the result was less than *P*-value 0.05, it was statistically significant.

## RESULTS

The preoperative demographics of the study population are described in Table 1. A total of 124 eyes of 124 participants (32 male and 92 female) were registered. The mean age of subjects was 28.92±6.33 (range, 26-42 years). Before the instillation of the ophthalmic solutions, all ocular examinations from all subjects, including TBUT (4.80±2.14s), the corneal staining score (0.89±0.70), meibomian gland expressibility (1.60±0.82), Meiboscore (0.78±0.66), OSDI score (33.67±19.11), showed DED status of participants.

**Changes of Average Lipid Layer Thickness** Figure 2 showed that change of LLT measured by LipiView interferometer. Mean baseline LLT before ophthalmic solutions measured by Lipiview interferometer were 61.50±2.57 for diquafosol, 62.23±2.64 for normal saline, 62.80±3.03 for hyaluronate, 62.96±3.25 for gatifloxacin. Mean changes of LLT after instillation (nm, mean value ± standard error) were 73.27±2.67 for diquafosol ($P$<0.001), 64.38±2.81 for normal saline ($P$=0.301), 64.28±3.17 for hyaluronate ($P$=0.495), 63.48±3.34 for gatifloxacin ($P$=0.884; Table 2). LLT after instillation of diquafosol was significantly increased (Figure 3).

**Table 1 Demographics of the subjects**

| Parameters | Data |
|---|---|
| Age, y | 28.92±6.33 (range, 26-42y) |
| Sex | 32 males and 92 females |
| Best corrected visual acuity | 0.16±0.08 logMAR |
| TBUT | 4.80±2.14 |
| Corneal staining score | 0.89±0.70 |
| Meibomian expressibility | 1.60±0.82 |
| Meiboscore | 0.78±0.66 |
| OSDI score | 33.67±19.11 |

TBUT: Tear break-up time.

**Table 2 Changes of LLT measured by LipiView interferometer**

| Treatment group | Baseline | After | $P^a$ |
|---|---|---|---|
| Diquafosol eye drop 3% | 61.50±2.57 | 73.27±2.67 | <0.0001 |
| Isotonic normal saline | 62.23±2.64 | 64.38±2.81 | 0.3013 |
| Gatifloxacin 0.3% | 62.96±3.25 | 63.48±3.34 | 0.8844 |
| Hyaluronate 0.1% | 62.80±3.03 | 64.28±3.17 | 0.4954 |

LLT: Lipid layer thickness. [a]Paired *t*-test.

## DISCUSSION

Diquafosol eye drop contains diquafosol sodium which is an agonist of $P2Y_2$ receptor as the active component. The $P2Y_2$ receptors are widely distributed in various organs throughout the body. Adenosine triphosphate (ATP) or uridine triphosphate

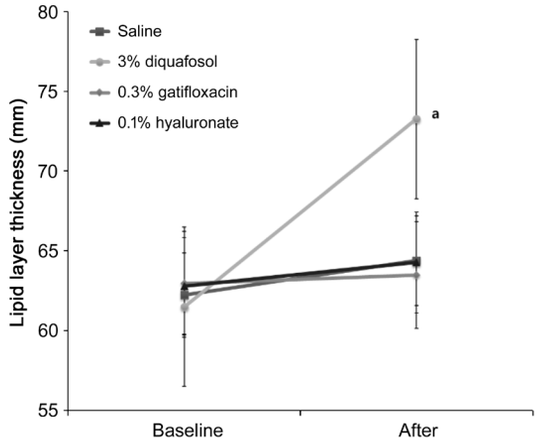


**Figure 3 Mean changes of LLT measured with LipiView interferometer** [a]Statistically significant in 3% diquafosol group ($P$<0.05).

(UTP) conduct as endogenous agonists of the $P2Y_2$ receptor. The $P2Y_2$ receptor is existed in the cornea, conjunctival goblet cells, and meibomian gland onto the ocular surface[9], and it has been known that ATP and UTP induce aqueous component and mucin secretion in the conjunctival tissue[18-20]. Diquafosol sodium which is a dinucleotide derivative as an agonist of $P2Y_2$ receptor[21], promotes fluid transport from the serosal to mucosal (tear film) side through chloride ion channel activation after elevating intracellular calcium ion concentration in the conjunctival epithelium[6,18]. In addition, diquafosol sodium activates the expression of membrane-associated mucin on the corneal epithelium and conjunctival goblet cells, induces the mucin secretion[22]. Previous study with healthy subjects showed that diquafosol eye drop increased tear film volume for up to 30min[23]. Therefore, these effects of diquafosol eye drop induce the tear film stability and relief for ocular symptoms. Previous clinical studies showed the efficacy of 3% diquafosol eye drop for the treatment of DED[24-25].

Yokoi *et al*[23] reported for the first time in human eyes that a single topical eye drop of 3% diquafosol induced significant increasing of the tear volume. In past prospective clinical study[26] indicated that 3% diquafosol eye drop are effective in improving both subjective symptoms and objective TBUT in patients with short TBUT. A study of 14 female DED with Sjögren's syndrome, 3% diquafosol eye drop may have efficacy for mild to moderate DED patients[27].

The activation of the $P2Y_2$ receptor to increase tear film lipid component on ocular surface is less well studied in human species, but previous animal experiments proved the expression of $P2Y_2$ receptor in the meibomian gland[9-10].

Obstructive meibomian gland dysfunction (MGD) which is the major cause of evaporative DED, is represented stagnation of meibum secretion and it may or may not be accompanied by a quantitative or qualitative changes in meibum[28-29]. Hyposecretion of tear film lipid component might result in short TBUT, tear film instability, and aggravation symptoms of DED. The change of meibomian gland directly affects LLT and tear stability. In this study, we found that the meibomian dysfunction affects dry eye syndrome, as seen in meibomian expressibility and meiboscore.

Arita *et al*[30] investigated the treatment effect of 3% diquafosol eye drop for DED with obstructive MGD. Diquafosol 3% treatment was applied with 4 times daily, and clinical follow-up period persisted at least 4mo (range 4-16mo). Subjective symptoms, ocular surface inflammation, meibomian gland status, TBUT, and tear meniscus height was improved in all 19 eyes of 10 patients. This study analyzed with quantitative noncontact meibography, showed a significant improvement in meibomian gland coverage from 36.9%±10.1% to 41.5%±9.2% after 3% diquafosol eye drop treatment ($P$<0.0001). This study suggests that 3% diquafosol eye drop might act on stimulating the $P2Y_2$ receptor *via* the meibomian gland ductal epithelium.

Our study showed a statistically significant increase in of LLT after 3% diquafosol eye drop instillation more than other control ophthalmic solutions. Fukuoka and Arita[31] was proved that a single topical eye drop of 3% diquafosol increase significantly LLT in the healthy human eye. Participants showed TBUT, corneal staining score was slightly decreased, ocular surface inflammation was not severe dry eye status. Most participants showed a mild obstructive MGD pattern with a decrease in meibomian gland expressibility and some damage on meibograpy. This study is meaningful as that first paper demonstrated through quantitative test it in dry eye patients with control ophthalmic solutions. We thought that this study is significant as the research that will support the result of the previous studies[30-31].

LipiView interferometer could measure meibomian gland of the upper and lower lid. The lower eyelid is easy to evaluate better than the upper eyelid by LipiView interferometer. In order to evaluate meibomian gland from the upper eyelid, the eyelids must be eversion, which in turn pushes the upper eyelid, which could lead to errors in the LLT and affect the measurement of other parameters of DED. Meibomian gland loss in upper eyelid was correlated with those in the lower eyelid, and both were correlated with changes of LLT in the obstructive MGD patients. Thus, a single measurement of LLT and meibography in lower eyelid might be enough for the assessment of MGD status, not necessarily measuring both eyelids[32]. LLT has negative correlation with obstructive MGD in upper and lower eyelid[14]. Increasing of LLT by 3% diquafosol ophthalmic solution could be a therapeutic effect on the MGD.

There are two possible mechanisms. First, $P2Y_2$ receptors on the ocular surface and the inner surfaces of the eyelids activated by diquafosol tetrasodium, promotes the natural

tear secretion. It causes ocular surface hydration through the release of electolyte, water, mucin and other components of the tear film[7,33]. Cowlen *et al*[9] proved the mRNA expression $P2Y_2$ receptor in the corneal, conjunctival epithelium and goblet cell, and meibomian gland ductal epithelial cells using in situ hybridization analyses in the rabbit and rhesus macaque. Tanioka *et al*[10] showed that $P2Y_2$ receptor expression was existed in the meibomian gland and lacrimal gland ductal epithelium using the immunohistological examinations. Our study suggests that diquafosol tetrasodium also directly activates $P2Y_2$ receptors on the meibomian gland of the eyelids and leads to an increase in the meibomian glands secretions. In animal experiments performed by the mouse experiment, Fujihara *et al*[33] the area of PAS-stained conjunctival goblet cells decreased significantly from 5min after 3% diquafosol eye drop instillation, and after 25min the cell area was normal. Diquafosol instillation significantly increased sialic acid, a marker of mucin-like substances, although the effect was transient at 5min (but not at 20min)[34]. Mucin secretion is known to be induced rapidly from 5min after instillation. It is known that the increase in LLT is maintained from 15min to over 60min after diquafosol eye drop instillation in healthy human eyes[31]. In this study, the time (20min after diquafosol instillation) of the increasing LLT after 3% diquafosol eye drop instillation was longer than that in increasing tear volume or sialic acid concentration. Thus, it suggests that the increasing of the tear film lipid layer actually induced by diquafosol eye drop instillation and it is thought to have worked independently of the role associated with mucin secretion.

And diquafosol increase water and mucin component on tear film through activation of $P2Y_2$ receptors and it may improve the tear film stability. Mucin is high-molecular weight glycoproteins with a protein backbone and a high carbohydrate component. Mucin has one important role which makes the tear film hydrophilic status. Mucin decreases ocular surface tension and induced evenly stable tear film, allowing the aqueous component of tear film to spread over the ocular surface. In animal studies, immediate effects after diquafosol instillation showed increased corneal wettability and increased MUC5AC concentration in the tear, which is known to increase tear film stability by increasing corneal wettability[35]. Increased tear stability and decreased surface tension of tear film will result in a redistribution of the lipid components secreted by the meibomian gland. This is as a result leads to an increase in overall LLT. Previous studies have shown that an increase in tear film lipid layer can be seen after artificial tear drops[36]. This study also showed that instillation of artificial tears induces redistribution of tear components, resulting in a temporary increase in tear file lipid layer as measured by Lipiview.

There were some limitations in this study. First, this study did not evaluate whether the increase in LLT was maintained during clinical follow-up. However, it is meaningful that the increase of LLT after the use of diquafosol was quantitatively evaluated using a LipiView interferometer in human patients. Second, the patients were limited to patients with DED with mild obstructive MGD. We need to study patients with different types of MGD (like hyposecratoy and hypersecretory) in the same study design. Third, this study did not evaluate the changes of subjective DED symptoms correlated with changes in LLT. The study of the relationship between the symptoms of patients with increased LLT will be needed. Fourth, study participants are limited to younger age groups (26-42 years old). Although there is an advantage of eliminating other age-related anterior segment diseases, further studies are needed to see whether the same results are obtained in the elderly.

In conclusion, diquafosol causes lipid layer reinforcement, as well as aqueous and mucin layer of the tear. Therefore, it is considered to be useful treatment option at evaporative dry eye with meibomian dysfunction, as well as aqueous deficient dry eye. Diquafosol eye drop might be the treatment options for MGD that have poor response to conventional treatments, such as warm compression, lid scrub, and using artificial tear or systemic anti-inflammatory medications.

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**Conflicts of Interest: Kang DH,** None; **Lee YW,** None; **Hwang KY,** None; **Koh KM,** None; **Kwon YA,** None; **Kim BY,** None; **Song SW,** None; **Kim KY,** None.

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