A topical eye drop versus intra-meibomian gland injection of bevacizumab for meibomian gland dysfunction patients.

Miss Chitchanok Tantipat

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การศึกษาเปรียบเทียบประสิทธิภาพระหว่างการหยอดตาและการฉีดยาบีวาซิซูแมบ (bevacizumab) เข้าสู่ต่อมไขมันที่เปลือกตาในการรักษาผู้ป่วยโรคต่อมไขมันที่เปลือกตาอุดตัน

น.ส.ชิดชนก ตันติพัฒน์

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คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	A topical eye drop versus intra-meibomian gland injection of bevacizumab for meibomian gland dysfunction patients.
By	Miss Chitchanok Tantipat
Field of Study	Clinical Sciences
Thesis Advisor	Associate Professor KRIT PONGPIRUL, M.D., Ph.D.
Thesis Co Advisor	Associate Professor NGAMJIT KASETSUWAN, M.D.

Accepted by the FACULTY OF MEDICINE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF MEDICINE
(Professor SUTTIPONG WACHARASINDHU, M.D.)

THESIS COMMITTEE

..... Chairman
(Professor WASEE TULVATANA, M.D.)

..... Thesis Advisor
(Associate Professor KRIT PONGPIRUL, M.D., Ph.D.)

..... Thesis Co-Advisor
(Associate Professor NGAMJIT KASETSUWAN, M.D.)

..... Examiner
(Assistant Professor WANNARASMI KETCHART, M.D., Ph.D.)

..... External Examiner
(Professor SUTHILUK PATUMRAJ, Ph.D.)

ชิดชนก ตันติพัฒน์ : การศึกษาเปรียบเทียบประสิทธิภาพระหว่างการหยอดตาและการฉีดยาบีวาซิซูแมบ (bevacizumab) เข้าสู่ต่อมไขมันที่เปลือกตาในการรักษาผู้ป่วยโรคต่อมไขมันที่เปลือกตาอุดตัน. (A topical eye drop versus intra-meibomian gland injection of bevacizumab for meibomian gland dysfunction patients.) อ.ที่ปรึกษาหลัก : รศ. ดร. นพ.กฤษณ์ พงศ์พิรุฬห์, อ.ที่ปรึกษาร่วม : รศ. พญ.งามจิตต์ เกษตรสุวรรณ

วัตถุประสงค์: เพื่อศึกษาเปรียบเทียบประสิทธิผลและความปลอดภัยระหว่างการรักษาด้วยการหยอดตากับการฉีดยาบีวาซิซูแมบเข้าสู่ต่อมไขมันที่เปลือกตาเมื่อใช้ร่วมกับการทำความสะอาดเปลือกตาแบบมาตรฐานในผู้ป่วยโรคต่อมไขมันที่เปลือกตาอุดตัน วิธีการวิจัย: การศึกษานี้เป็นการศึกษาทางคลินิคเปรียบเทียบแบบสุ่ม โดยผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรคต่อมไขมันที่เปลือกตาอุดตันร่วมกับพบภาวะเส้นเลือดที่ขอบเปลือกตาที่ผ่านการคัดเลือกจะได้รับการแบ่งกลุ่มโดยวิธีการสุ่มเป็น 2 กลุ่มคือ กลุ่มแรกได้รับการรักษาด้วยยาหยอดตาบีวาซิซูแมบวันละ 4 ครั้งทั้ง 2 ข้างร่วมกับการประคบอุ่นและทำความสะอาดเปลือกตา กลุ่มที่ 2 ได้รับการฉีดยาบีวาซิซูแมบเข้าสู่ต่อมไขมันที่เปลือกตาทั้ง 2 ข้างจำนวน 1 ครั้งร่วมกับการประคบอุ่นและทำความสะอาดเปลือกตา ผลลัพธ์หลักที่ต้องการศึกษาคือ ปริมาณเส้นเลือดที่ขอบเปลือกตา ซึ่งสามารถวัดได้ 2 วิธีคือการประเมินโดยจักษุแพทย์และการคำนวณด้วยคอมพิวเตอร์ ผลลัพธ์อื่นๆ ได้แก่ อาการตาแห้ง การตรวจการติดสีฟลูออเรสซีนของผิวกระจกตา การตรวจคุณภาพของน้ำมันที่เปลือกตา การตรวจปริมาณความเสื่อมของต่อมไขมันที่เปลือกตาจากการถ่ายรูป ความแดงของเยื่อบุตาขาว เวลาในการคงสภาพของชั้นน้ำตาจากการตรวจด้วยการย้อมสีฟลูออเรสซีนและการถ่ายภาพ และการตรวจด้วยการถ่ายภาพความหนาของชั้นไขมันในชั้นน้ำตา ความสม่ำเสมอในการประคบอุ่นและทำความสะอาดเปลือกตาและผลข้างเคียงจากการรักษาด้วยยาบีวาซิซูแมบ เป็นต้น อาสาสมัครจะได้รับการตรวจติดตามที่สัปดาห์แรก เดือนที่ 1, 2 และ 3 หลังการรักษา ผลการศึกษา: อาสาสมัคร 30 คนได้รับการสุ่มเพื่อรับยาหยอดจำนวน 15 คน และรับการฉีดยาบีวาซิซูแมบจำนวน 15 คน ในกลุ่มฉีดยาพบว่าปริมาณเส้นเลือดที่ขอบเปลือกตาซึ่งวัดด้วยการประเมินและการคำนวณด้วยคอมพิวเตอร์มีค่าลดลงอย่างมีนัยสำคัญที่ 3 เดือนหลังการรักษา ($p<0.05$) ในขณะที่กลุ่มหยอดยาไม่พบการเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติเมื่อวัดปริมาณเส้นเลือดด้วยคอมพิวเตอร์ ในผลลัพธ์อื่นๆของการศึกษาพบว่ากลุ่มยาฉีดมีการเปลี่ยนแปลงที่ดีขึ้นอย่างมีนัยสำคัญทางสถิติในค่าการติดสีฟลูออเรสซีนของผิวกระจกตา ค่าคุณภาพของน้ำมันที่เปลือกตา ค่าปริมาณความเสื่อมของต่อมไขมันที่เปลือกตาจากการถ่ายรูป เปรียบเทียบกับกลุ่มหยอดยาอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในขณะที่ทั้งสองกลุ่มมีอาการตาแห้ง ค่าคุณภาพของน้ำมันที่เปลือกตา ค่าปริมาณความเสื่อมของต่อมไขมันที่เปลือกตาจากการถ่ายรูป ค่าการติดสีฟลูออเรสซีนของผิวกระจกตาและค่าความแดงของเยื่อบุตาขาว ที่ดีขึ้นอย่างมีนัยสำคัญทางสถิติเปรียบเทียบก่อนและหลังการรักษาที่ 3 เดือน ($p<0.05$) ทั้งสองกลุ่มไม่พบผลข้างเคียงที่ร้ายแรงหลังการรักษาด้วยยาบีวาซิซูแมบ สรุปผลการศึกษา: ทั้งสองวิธีของการให้ยาบีวาซิซูแมบด้วยการหยอดตาและการฉีดยาเข้าสู่ต่อมไขมันที่เปลือกตาร่วมกับการทำความสะอาดเปลือกตาแบบมาตรฐานมีความปลอดภัยและมีประสิทธิภาพในการลดปริมาณเส้นเลือดที่ขอบเปลือกตาและลดอาการแสดงต่างๆในผู้ป่วยโรคต่อมไขมันที่เปลือกตาอุดตัน ดังนั้นการรักษาด้วยยาบีวาซิซูแมบทั้ง 2 วิธีสามารถใช้เป็นทางเลือกหนึ่งหรือเป็นการรักษาร่วมกับการรักษามาตรฐานของผู้ป่วยโรคต่อมไขมันที่เปลือกตาอุดตันได้

สาขาวิชา	เวชศาสตร์คลินิก	ลายมือชื่อนิสิต .....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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KEYWORD: meibomian gland dysfunction (MGD) lid margin telangiectasia bevacizumab vascular endothelial growth factor (VEGF) lid hygiene

Chitchanok Tantipat : A topical eye drop versus intra-meibomian gland injection of bevacizumab for meibomian gland dysfunction patients.. Advisor: Assoc. Prof. KRIT PONGPIRUL, M.D., Ph.D. Co-advisor: Assoc. Prof. NGAMJIT KASETSUWAN, M.D.

Purpose: To compare the efficacy and safety of topical bevacizumab eye drop versus intra-meibomian gland injection of bevacizumab when used with the standard lid hygiene in meibomian gland dysfunction (MGD) patients.

Methods: 60 eyes of 30 MGD patients with lid margin telangiectasia were randomized to receive 0.05% bevacizumab eye drop or single 2.5% intra-meibomian gland bevacizumab injection plus standard lid hygiene. The primary outcomes were telangiectasia grading and the computerized lid margin neovascularized area (LMNA). The secondary outcomes were the ocular surface disease index (OSDI) score, corneal staining, meibomian gland quality, meiboscore, conjunctival redness, fluorescein break up time (FBUT), noninvasive tear breakup time (NIBUT), lipid layer thickness (LLT), compliance of treatments, and adverse events (AE). All the parameters were re-evaluated before and until 3 months after treatment.

Results: A significant improvement in telangiectasia grading and LMNA, primary outcomes, were observed in injection group at month 3 ($p<0.05$) but LMNA was not apparent in the eye drop group. In the injection group, there were significant improvements in corneal staining, meiboscore, and FBUT compared with the eye drop group ($p<0.05$). Both groups showed significant improvements in OSDI score, corneal staining, MG quality, meiboscore, and conjunctival redness compared with pre-treatment. ($p<0.05$).

Conclusions: Both routes of intra-MG injection and eye drop bevacizumab administrations were safe and effective in reducing lid margin telangiectasia and signs and symptoms of MGD. Therefore, both routes of administration could be an alternative or adjunctive treatment with the standard lid hygiene for MGD patients.

Field of Study: Clinical Sciences

Academic Year: 2020

Student's Signature .....

Advisor's Signature .....

Co-advisor's Signature .....

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Chapter 1 Introduction

Background and rationale

Dry Eye Disease (DED) is one of the common eye diseases in Thailand[1] and around the world. DED is caused mainly by Meibomian Gland Dysfunction (MGD).[2] Characteristics of MGD comprise chronic abnormality of the meibomian glands and alteration of gland secretion quality, which leads to tear film instability. Clinical signs of MGD are usually confined to the posterior lid margin. The signs include lid margin irregularity, prominent telangiectatic blood vessels coursing from outer to inner part of orifice, hyperplasia/metaplasia and pouting of the MG orifices.

Prevalence of MGD varies among geographic regions and ethnic groups, but it seems to be highest among Asian people.[3] In Bangkok, Thailand, Lekhanont et al.[1] reported that the MGD hospital-based prevalence was as high as 46.2%. However, In 2010, Kasetsuwan et al.[4] conducted a population-based study in Romklao District, Thailand, and found that the prevalence rate of dry eye was at 14.2%.

The pathophysiology of MGD is numerous, one of which is inflammation. According to the in vitro study,[5] when human conjunctival epithelial and fibroblast cells are stimulated by the environment with increased inflammatory cytokines, cells will produce more vascular endothelial growth factors (VEGF). The result of the in-vitro study is similar to the study in humans.[6] The different inflammatory cytokines were compared between patients with mild to moderate severity of MGD and normal healthy volunteers. As a result, the first group possessed significantly higher levels of VEGF than the latter group, and the rise of VEGF was found to stimulate neovascularization, increase vascular permeability, and raise infiltration of inflammatory cells. Moreover, it is believed that VEGF is one of pro-inflammatory cytokines[7] which stimulates IL-6, IL-8, and TNF-α.

Telangiectasia or lid margin vascularity is a clinical sign that usually co-exists with MGD. It is the small superficial dilatation of conjunctival blood vessels around the lid margin, and it can be prevalently observed in normal elders[8] and MGD patients, especially patients diagnosed with MGD-related rosacea.[9] A population-based study in Taiwan reported that the prevalence of telangiectasia is as high as 70% in Chinese

patients aged more than 65 years.[10] It is assumed that the pathogenesis of telangiectasia comprises UV light, neurovascular, and neuroimmune dysregulation.[11] Furthermore, lid margin telangiectasia is one of the criteria for MGD diagnosis.

At present, the standard MGD treatment is warm compression and lid hygiene. It is discovered that most patients do not regularly follow the treatment process,[12] thus, the treatment result can be different from the expectation. Moreover, the regular standard warm compression and lid hygiene treatment may not help reduce lid margin telangiectasia.[13]

In some cases treated with standard lid hygiene, patients complain of increased lid tenderness. Steven L Maskin provided an explanation[14] that these patients may possess membrane or fibrosis obstruction of MG orifice, which needs mechanical probing. In addition, many kinds of efficient medication, such as topical steroid, are utilized in the treatment of MGD caused by inflammation. However, this can lead to a lot of side effects such as ocular hypertension and cataract. Hence, it is not advisable to instill such medication for a long period of time.

For years, bevacizumab (an anti-VEGF-A recombinant humanized monoclonal antibody) has been used widely in the treatment of systemic and eye diseases such as diabetic macular edema in diabetic retinopathy. VEGF-A is a main regulator of angiogenesis,[15] increase vascular permeability,[16] as well as chemotactic for macrophages,[17] whose role are to release VEGF-C and VEGF-D. VEGF-C and VEGF-D are another contributors to lymphangiogenesis,[17] which is one of the pathogenesis of DED,[18,19] and neurotrophic factor.[20] In 2012, Goyal et al.[21] found that anti VEGF-C treatment could alleviate DED in murine model by improving inflammation at the clinical and cellular levels. Another study[22] also revealed that VEGF-A level on mice skin increased after exposed to UVB radiation. However, VEGF-C and VEGF-D levels remained the same. The abnormality caused by VEGF-A included dilated, leaky, and poorly functional lymphatic vessels.

In one study[23] for MGD treatment with lid margin telangiectasia, bevacizumab was injected into meibomian gland, and it was found that, in 3 month period, lid margin

telangiectasia decreased as much as 42%, compared with the baseline for the study. In addition, it helped reduce MGD symptoms. In 2009, Koenig et al.[24] used 0.05% bevacizumab eye drops 5 times per day in patients diagnosed with corneal neovascularization from the second week to twelfth month of the treatment period. After the treatment, the vascularized areas and vessel diameters were reduced without serious side effects. Then, in 2020, Kasetsuwan et al.[25] used 0.05% bevacizumab eye drops to treat DED patients. During the third month of the treatment period, fluorescein break up time (FBUT) increased, staining was reduced, and dry eye symptoms improved significantly in the study group.

Based on previous studies, our research group creates an assumption that the treatment with topical or intra-meibomian gland (MG) injection of bevacizumab, together with standard lid hygiene, would help reduce lid margin telangiectasia and improve MGD signs and symptoms. According to the literature review, this study is considered the first trial to use bevacizumab in the form of eye drops to treat MGD. Furthermore, the treatment of lid margin telangiectasia will be assessed through the lid margin neovascularized areas (LMNA) which are measured with highly accurate computer image software analysis. The results will be compared before and after the treatment during the third month of the study.

Hypothesis

Null hypothesis

- The effect of topical bevacizumab plus standard lid hygiene in term of reduction of lid margin telangiectasia and improvement of MGD signs and symptoms in patient with MGD is not different from the effect of intra-MG injection of bevacizumab plus standard lid hygiene.

Alternative hypotheses

- The effect of topical bevacizumab plus standard lid hygiene in term of reduction of lid margin telangiectasia and improvement of MGD signs and symptoms in patient with MGD is superior to the effect of the intra-MG injection plus standard lid hygiene.

- The effect of topical bevacizumab plus standard lid hygiene in term of reduction of lid margin telangiectasia and improvement of MGD signs and symptoms in patient with MGD is inferior to the effect of the intra-MG injection plus standard lid hygiene.

Objectives

To compare the efficacy of topical eye drop versus intra-meibomian gland injection of bevacizumab when used with the standard lid hygiene in MGD patients

To compare the safety of topical eye drop and intra-meibomian gland injection of bevacizumab when used with the standard lid hygiene in MGD patients

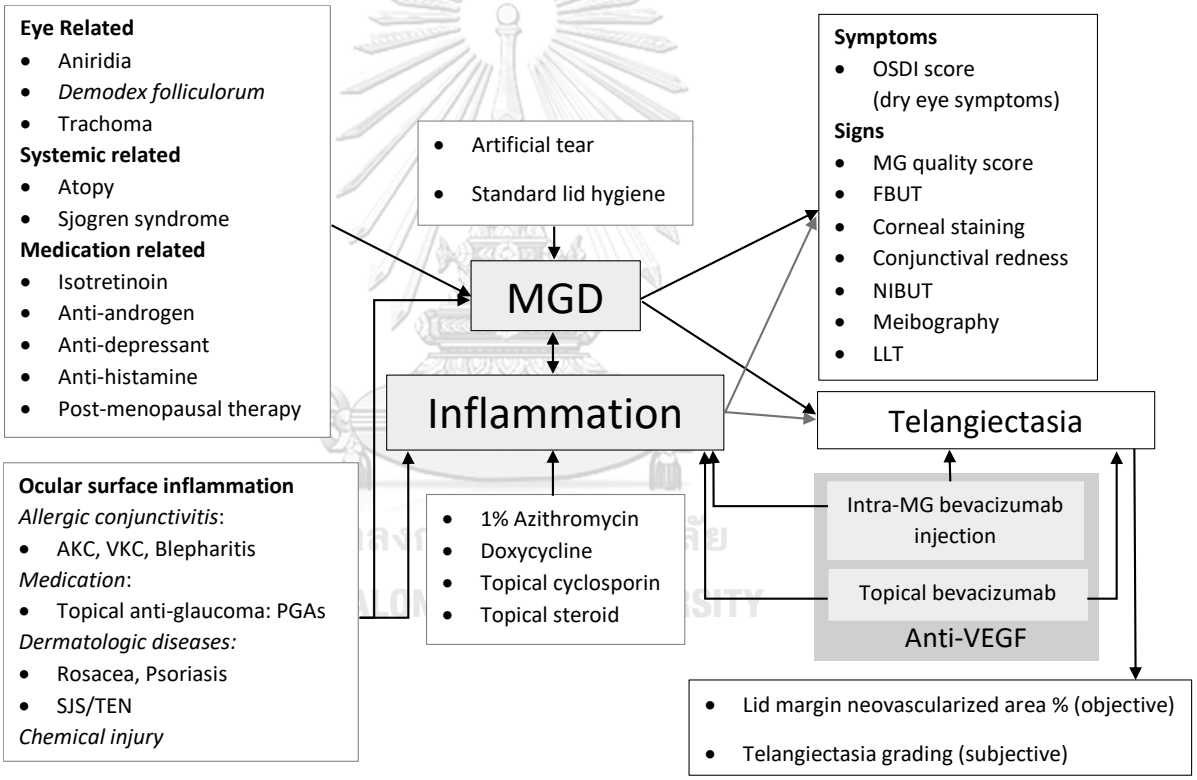


Figure 1 *Conceptual Framework*

Keywords

meibomian gland dysfunction (MGD), lid margin telangiectasia, bevacizumab, vascular endothelial growth factor (VEGF), lid hygiene

Chapter 2 Literature Review

Meibomian Gland Dysfunction (MGD) is a chronic abnormality of meibomian glands. Normally detected symptom is blockage of meibomian glands. This can be found together with the alteration of the quality and quantity of meibum, which is a component of the tear lipid layer. Lid margin abnormalities are comprised of lid margin irregularity, prominent, telangiectatic blood vessels, hyperplasia, metaplasia and pouting of the MG orifices. As a result, patients with MGD have the conditions of dry eyes, irritation, and inflammation of ocular surface and eyelid margin.

Besides MGD conditions, telangiectasia or lid margin vascularity can be normally found in MGD patients. Telangiectasia is the small superficial dilatation of blood vessels in conjunctiva around the lid margin, which is presumably caused by ultraviolet light [8](UV). It can be prevalently found in normal elders' lower eyelid margin.

According to the study conducted by Pflugfelder C S et al.[9] in 1998, telangiectasia significantly rose in patients with inflammatory MGD or MGD related rosacea, compared with ATD Sjogren patients and healthy volunteers. Its presentations included the exaggeration and invasion of the outer to the inner cuffs of orifice. Based on the pathophysiology of rosacea, it could be presumed that erythema and telangiectasia was initiated by neurovascular dysregulation and abnormal neuroimmune response.[11] At present, telangiectasia become one of the criteria for MGD diagnosis.

The standardized MGD treatment obtained from the international workshop on MGD[26] is warm compression and lid hygiene treatment, which is highly efficient. However, there are problems in such way of treatment. For instance, there is no clear standard of the treatment, duration, frequency, and heat applied in the treatment is not thorough nor constant. Apart from these problems, another difficulty is the standardized treatment requires various skills and a lot of time, thus, the treatment is not as effective as expected, and most patients do not follow its procedures.

In 2004, Romero J M et al.[13] evaluated the efficacy of lid hygiene and preservative-free artificial tears for MGD during a 6-week period. The results showed that this conservative treatment significantly improved TBUT and relieved the dry eye

symptoms, but there was no statistically difference between the slit-lamp photographs before and after treatment including lid margin telangiectasia.

As for pathological condition and mechanism of MGD,[27] MGD occurs from the clogging of the terminal ducts of meibomian glands, which may be the result of the inflammation of eyelids and ocular surface. Because of the obstruction, meibum cannot be released outside, and pressure in the gland would rise, leading to inflammation. In the end, if the clogged gland is not treated, meibomian glands will be atrophy. Lipid layer in the tear cannot be produced, resulting in evaporative dry eyes. Thus, MGD patients have dry eyes, eye discomfort, and blurred vision. Obviously, inflammation is part of the main mechanism of MGD, hence, medication used in inflammation treatment, such as steroid eye drop, is used to alleviate inflammation, leading to better conditions and symptoms. However, instilling eye drops for a long period of time or in patients with risk factors may result in side effects such as rising of intra-ocular pressure, causing glaucoma, and blurred vision from steroid induced cataracts.

At present, bevacizumab is widely employed and becomes standardized treatment for some eye diseases such as diabetic macular edema from diabetic retinopathy. Its mechanism of actions is that it is a VEGF-A antibody, with the effect of reducing vascular permeability, neovascularization, inflammatory cells infiltration, and pro-inflammatory cytokines. Bevacizumab is used to treat eye diseases in many forms such as intravitreal injection and subconjunctival injection. Examples of these methods of treatment are adjunctive subconjunctival bevacizumab injection with trabeculectomy, subconjunctival injection or eye drops instillation with pterygium excision, instilling eye drops to decrease corneal neovascularization after several kind of corneal diseases.[24]

According to the meta-analysis,[28] it is found that side effects from using bevacizumab in the forms of an eye drop and subconjunctival injection for are relatively safe so it can be utilized in treating patients diagnosed with ocular diseases.

For intra-MG drug delivery route, in 2011, Maskin L S et al.[29] reported the retrospective case series of MGD with lid tenderness treated with intraductal MG probing with adjunctive intraductal microtube for steroid injection. They discovered that

such treatment could reduce 94% of lid tenderness in the 1st- 3rd month after the treatment. The most common adverse event of this procedure is dot hemorrhages[14] at the orifices due to the relief of disorganized periductal fibrovascular scar. This condition is self-limited and does not require pressure or other treatment.

In 2012, Goyal S et al.[21] performed an experiment by injecting anti-VEGF in guinea pigs. It was discovered that the medication can treat dry DED in guinea pigs; it can significantly reduce dry eye symptoms, inflamed cells, and VEGF, compared with the group of guinea pigs injected with saline solution.

In 2016, Kwon J W et al.[30] carried out a similar study. He injected anti-VEGF, dexamethasone and saline solution in the conjunctiva of the group of guinea pig with dry eye condition and the controlled group. It was seen that, in the first group, anti-VEGF can greatly reduce neovascularization and inflammation better than the groups received dexamethasone and saline solution.

In 2015, Jiang X et al.[31] conducted a research by perform subconjunctival bevacizumab injection, at the amount of 25 mg/mL and 0.1 mL in total, in 64 eyes of dry eye patients. After monitoring for 3 months, it was discovered that there was a significant improvement in dry eye symptoms, TBUT, conjunctival vascularization area and the density of goblet cell after treatment compared to baseline ($p< 0.05$). There was no local and systemic side effect observed in any patient.

In the same year, Kasetsuwan N et al.[32] was successful at pharmaceutical bevacizumab preparation in the form of an eye drop. They conducted a study using 0.05% of bevacizumab eye drop to prevent the recurrence of pterygium after bare sclera excision technique. It was found that the possibility of reducing conjunctival and corneal recurrences in the experiment group was significantly more than the control group ($p=0.01$) at the period of 3 months after the surgery. There was no significant local and systemic side effects developed in association with instillation of topical bevacizumab.

In 2009, Koenig Y et al.[24] study the efficacy and safety of topical bevacizumab for treatment of corneal neovascularization secondary to a variety of corneal diseases. It

was found that 0.5% topical bevacizumab inhibit corneal neovascularization, and lead to a reduction in vascularized area for 61%, (p=0.0182) in the last follow-up group, a reduction of the vessel diameter for 24% after treatment, (p=0.01). For the safety, the results suggested that bevacizumab eye drop is relatively safe and well-tolerated medication for the treatment of corneal neovascularization but care should be cautious in patients with epithelial defects and neurotrophic keratopathy.

In 2018, Jiang X et al.[23] carried out a study by utilizing 25 mg/mL of intra-meibomian gland injection of bevacizumab, 0.15 mL in total, in 26 eyes of MGD patients with lid margin telangiectasia. It was seen that intra-meibomian gland bevacizumab injection significantly improved lid margin telangiectasia, conjunctival injection, MG quality, MG expressibility, TBUT, corneal staining and OSDI at 3 months compared to baseline, ($p<0.05$). No local and systemic side effects were observed at follow-up visits.

In 2020, Kasetsuwan N et al.[25] conducted a study using 0.05% bevacizumab eye drop for treatment of DED and reported a significant improvement of OSDI score, corneal staining, and FBUT in DED patients treated with 0.05% bevacizumab eye drop at month 3 compared with the control group.

Chapter 3 Material and Methods

The study was conducted at Chula Refractive Surgery Center, King Chulalongkorn Memorial Hospital (KCMH) from September 2020 to May 2021 and performed under the approval of the Institutional Review Board (COA No. 947/2020) and the tenets of the Declaration of Helsinki. The Thai Clinical Trial Registry (TCTR) number is TCTR20201102001.

This study was primarily supported by the Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University [grant number RA63/094]. The funding did not involve in conducting this research.

Research design

Single center, open-label, observer-blinded, randomized controlled trial

Research Methodology

Population

MGD patients who come to outpatient clinic at Ophthalmology department of King Chulalongkorn Memorial Hospital

Table 1 MGD Staging

Stage	Symptoms	Clinical signs	Meibum quality	Meibum expressibility	Oxford staining
1	No discomfort, itching or photophobia	Based on gland expression	2 to <4	1	No staining
2	Mild symptoms of ocular discomfort, itching or photophobia	Scattered lid margin features	4 to <8	1	Oxford grade 0-3
3	Moderate symptoms of ocular discomfort, itching or photophobia with limitations of activities	Plugging, vascularity	8 to <13	2	Oxford grade 4-10
4	Marked symptoms of ocular discomfort, itching or photophobia with definite limitations of activities	Dropout, displacement	≥ 13	3	Oxford grade 11-15

(modified from MGD workshop)[26]

Table 2 *Meibum Quality Score and Expression Score*

8 glands in central third of lower eyelid	
Grade	Quality of meibum secretion
0	clear
1	cloudy
2	cloudy with granular debris
3	thick, like toothpaste

5 glands in central third of lower eyelid	
Grade	Number of expressible glands
0	all
1	3-4
2	1-2
3	0

(modified from MGD workshop)[26]

Table 3 *Telangiectasia Grading*[33]

Grade	Definition of lid margin telangiectasia
0	No or slight redness of lid margin conjunctiva No telangiectasia crossing MG orifices
1	Redness of lid margin conjunctiva No telangiectasia crossing MG orifices
2	Redness of lid margin conjunctiva Distribution of telangiectasia crossing MG orifices < half of total lid margin length
3	Redness of lid margin conjunctiva Distribution of telangiectasia crossing MG orifices ≥ half of total lid margin length

Target population

MGD patients with lid margin telangiectasia who receive topical bevacizumab plus standard lid hygiene

Control population

MGD patients with lid margin telangiectasia who receive intra-MG injection of bevacizumab plus standard lid hygiene

Approach to participant

Direct recruitment of potential study participants, referrals from non-investigator healthcare providers, information sheets, notices, advertisements

Inclusion criteria

- Age 18-80 years

- Symptoms ≥ 1: dryness, FB sensation, burning, tearing & duration > 6 months
- Diagnosis of MGD stage 2 or 3 with lid margin telangiectasia grade 2 or 3 both eyes
- Willingness to regular follow-up as appointed

Exclusion criteria

- Ocular structure abnormality
- History of ocular trauma, ocular and other surgery
- Use of any treatment for DED or MGD except artificial tears within the past month
- Active allergy, infection, inflammation at ocular surface unrelated to DE, MGD
- History of ocular herpes infection
- Lacrimal gland drainage system abnormality
- Contact Lens wear within the past month
- Use systemic medication affecting the ocular surface, systemic anti-inflammatory medication, anticoagulant or antiplatelet medication
- Unstable systemic diseases: uncontrolled HT, uncontrolled DM, stroke, coronary artery disease, cerebrovascular disease, bleeding diathesis
- History of bevacizumab contraindication: congestive heart failure, GI perforation, pregnancy, breast feeding, reversible posterior leukoencephalopathy syndrome (RPLS), proteinuria, surgery and wound healing complications
- Allergy to bevacizumab, moxifloxacin

Informed consent process

The research physician explained details regarding the inform consent process at the Chula Refractive Surgery Center, Ophthalmology Department of King Chulalongkorn Memorial Hospital. Such details comprised of explanations, objectives,

practice guidelines, benefits and risks towards participants. Consent document and information sheets were provided and participants' understanding was evaluated. In addition, the research physician answered all of the queries raised by the participants, and provided time for them to make an independent decision before signing the consent form to participate in the research.

Recruitment

Patients, who learned about this study from different channels (i.e. bulletin boards in hospitals, referral from other general ophthalmologists, or other potential studies) and were interested to participate, would be contacted to inquire about their attentions, make appointments, explain and clarify the information about the study. Subsequently, examiners would take histories from patients and perform eye assessment to determine whether the patients pass the inclusion and exclusion criteria. Details of the histories include the conditions of dry eye diseases, underlying conditions, and medications. During the study, participants had the right to leave the study at any time and were not required to provide their reason.

Random allocation

Patients were allocated into 2 groups by computer-generated block of 4 design randomization, which the allocation sequence was concealed by an independent third party.

Group 1: Intra-MG bevacizumab injection + standard lid hygiene

Group 2: Bevacizumab eye drop + standard lid hygiene

- All subjects will be instructed to perform the standard lid hygiene on both eyes 2 times/day while participating in this study.
- Subjects in group 1 will receive the intra-MG injection of bevacizumab for both eyes at the 1st day of joining the study.
- Subjects in group 2 will receive the topical bevacizumab for both eyes 4 times/day while participating in this study.

The outcomes will be collected at 1st visit, 1st week, 4th week, 8th week and 12th week after treatment. All clinical measurements were performed in both eyes by a single blinded investigator.

Blinding

Member of research	Blind	Discussion
1. Surgeon (1)	✗	- Not involved in follow up and assessment
2. Investigator (1)	✓	
3. Participants (30)	✗	- 1° outcome is objective measurement
4. Technician (1, analyze 1°outcome)	✓	
5. Pharmacologist (1)	✗	- Not involved in follow up and assessment

Intervention and control groups

Standard lid hygiene procedure

- Application of a warm towel to compress the eyes for 5 minutes.
- Lid massage was done by applying pressure with a finger or cotton bud toward lid margins with warm water or baby shampoo.
- Wash their lids with clean water to remove debris.
- Dry with a clean towel.

This method will be done at least twice daily while participating in the study

2.5% Intra-meibomian gland injection *(Figure 2)*

- One time, at the 1st visit
- Dose: 2.5 mg/0.1 mL total 150 μL, prepared from IV form under laminar flow at hospital pharmacy department and bevacizumab was ported into 1 mL syringes for daily use and store at 4°C during use
- 30-gauge needle with syringe 1 mL

- 10 % povidone iodine was applied on skin for 3 minutes then wipe off with NSS
- Tetracaine eye drop was applied to conjunctival sac and 4% lidocaine gel was directly applied with sterile cotton-tipped applicator to lid margin
- Contact lens was placed on cornea
- Intra-MG injection was pointed at an acute angle to skin near duct or around the duct which presented with the dense telangiectasia, depth 1-2 mm, 5 sites per eye (3 sites at upper and 2 sites at lower eyelid margin for total 150 μL)
- Done by expert surgeon (Dr. N.K.)
- 1 drop of 0.5% moxifloxacin was instilled
- Place: OR minor, Chula refractive surgery center

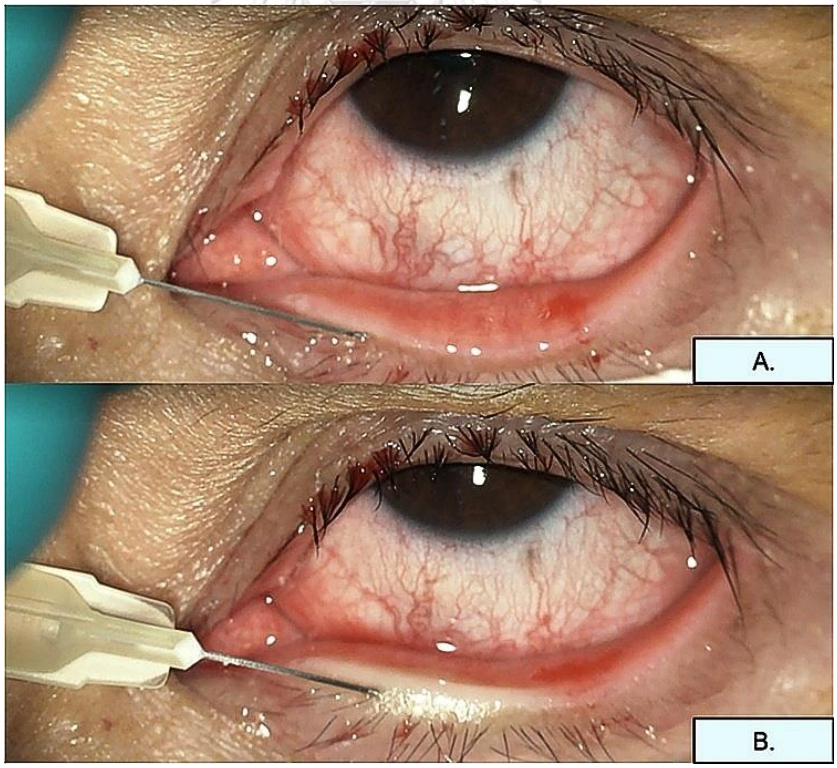


Figure 2 *Intra-Meibomian Gland Injection*

A*: Lower eyelid margin injection site*

B*: The lower eyelid margin turns white while injecting*

0.05% Bevacizumab eye drop

- Dose: 0.05 mg/0.1mL, prepared from IV form diluted in NSS under laminar flow at hospital pharmacy department, stored at -20°c until use, bevacizumab was ported into 5 mL eye dropper bottles for daily use and store at 4°C during use
- Drop 4 times/day for 12 weeks
- Store at 4°C during use and use within 2 weeks

Sample size calculation

To ensure an adequate sample size, we used the results from Dasjerdi et al.,[34] which reported the efficacy of 1% bevacizumab eye drop by assessing corneal neovascularized area and the results from Jiang's research[23] which reported the efficacy of 2.5% bevacizumab intra-MG injection by assessing lid vascularity. By using the formula to compare mean values between independent subjects, considered p-value of 0.05 to be statistical significance and 80% to be the study power, the calculated sample size was 12 patients per group. Finally, after adjusted for 20% drop out rate, the sample size was 15 patients per group.

- μtrt: Mean in a treatment group = 29.00
- σtrt: SD. in a treatment group = 10.00
- μcon: Mean in a control group = 42.00
- σcon: SD. in a control group = 10.00
- Ratio (control/treatment) = 1.00
- Zα = type I error, α = 5%; Z1-α/2 = 1.96
- Zβ = type II error, β = 10%; Z1-β = 0.84
- Adjusted drop out rate 20%
- Sample size: Treatments = 15, Controls = 15

Formula

$$n_{trt} = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \left[\sigma_{trt}^2 + \frac{\sigma_{con}^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_{con}}{n_{trt}}, \Delta = \mu_{trt} - \mu_{con}$$

Data collection

Demographic data will be collected by accessing to patients'OPD card and directly ask. Patient's symptoms will be evaluated by using OSDI questionaire. Other baseline and follow-up outcomes will be measured under slit-lamp biomicroscopic examination and specific MGD devices such as LipiView® and Keratograph 5M (Oculus®). Data will be collected at first visit, 1st week, 4th week, 8th week and 12th week after treatment. At post-operative intra-MG injection day 1, All participants will have an appointment for assessing the post-operative complication.

Outcome measurements

Ocular Surface Disease Index (OSDI) score: 12 questions, 0-100

Primary outcomes: lid margin telangiectasia

- Telangiectasia grading: 0-3
- Lid margin neovascularized area (LMNA): %

Slit-lamp photograph → LMNA image by image analysis software ***(Figure 3)***

- mean of 3 measurements
- how to optimize quality of photograph

We use the same Topcon slit lamp biomicroscopy, magnification x 10, steady head position (chin in chin rest, forehead at forehead band, eye look straight), take 3 photographs, in the same light condition, at the room number 2, Chula Refractive Surgery Center.

- image analysis software[24] (Cell Sens Dimension software®: Olympus, Hamburg, Germany)

- draw fixed region of interest (ROI): length x height (pixels)
  - length: the highest point of meibomian line extended 1/6 to the left and right of the total length of each picture
  - height: 1/6 of the total length of each picture
- the intensity and contrast of lid margin telangiectasia images were adjusted and neovascularized areas inside the ROI were calculated into pixels
- neovascularized areas were divided by ROI and calculated into % of LMNA
- analyzed by the blinded single outsource technician.

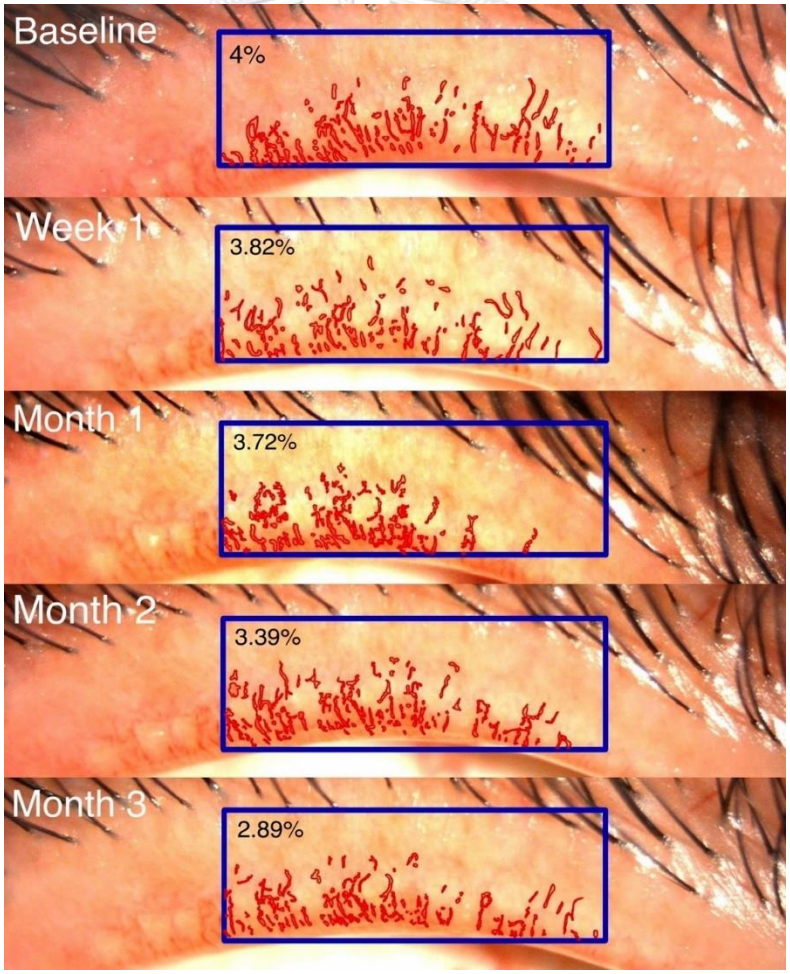


Figure 3 *Lid Margin Neovascularized Area Image (LMNA)*

Secondary outcomes: symptoms and signs of MGD

- OSDI score: 0-100

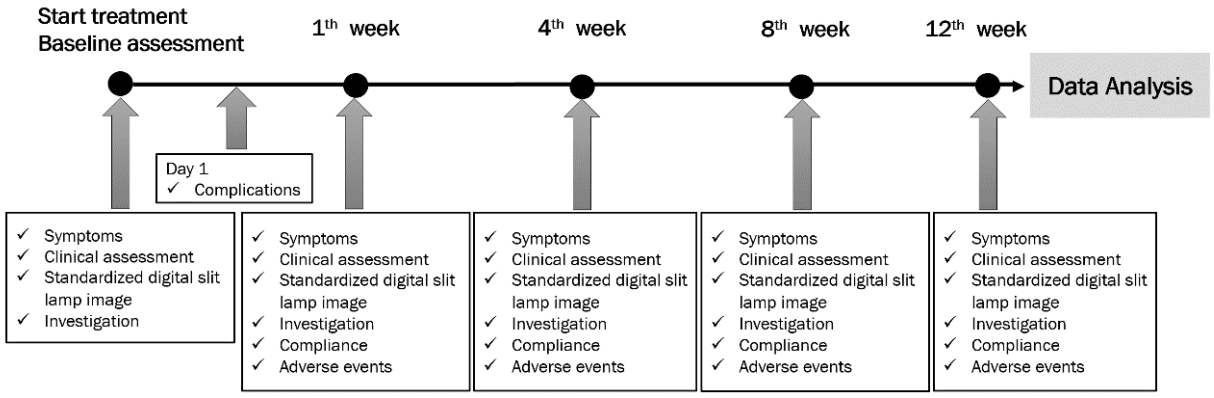
- MG quality score: 0-24
- Tear break up time (TBUT): seconds, mean of 3 measurements
- Corneal staining: Oxford grading scale,[35] 0-5
- Conjunctival redness: 0-4
- Lipid layer thickness (LLT): nanometers, LipiView® instrument (TearScience® Inc., Morrisville, NC, USA)
- Meibography score: 0-6, Keratograph 5M (Oculus®)
- Noninvasive tear breakup time (NIBUT): seconds, Keratograph 5M (Oculus®)

Adverse events:

- Local and systemic AEs of intra-MG injection, topical eye drop

Compliances:

- Frequency of perform standard lid hygiene/week: No./week
- Frequency of topical eye drop/day: No. of drop/day
- Frequency of bevacizumab eye drop/day: No. of drop/day



Ethical consideration

The proposal was submitted to the Institutional Review Board on Human Research at the Faculty of Medicine, Chulalongkorn University. A consent form was given before performing an intra-MG bevacizumab. The inform consent form was attached at the end of this proposal. All enrolled patients in this study were instructed how to perform the

standard warm compression and lid hygiene by watching the video demonstration and then the patients were randomized into 2 group of interventions.

Respect for person – provided information completely and answered all queries until the research participants clearly understood all of the details and independently made the decision in giving a consent to participate in the research. The researcher respected the privacy and maintained confidentiality of information collected from research participants.

Beneficence and non-maleficence – the benefits of research participants include warm compression and lid hygiene for MGD, which is a standard treatment that is currently accepted and provided the effective results. In addition, the research participants will receive the meticulous ophthalmic examination through a standardized digital slit-lamp biomicroscopy and high technological devices for assessment meibomian gland function. Minor risks towards the research participants include side effects from the use of topical and the intra-MG injection of bevacizumab which are irritation, epithelial defect, mild hemorrhage at injected site which resolved in the following day. Other less common systemic side effects include uncontrolled hypertension. Meanwhile side effects that are considerably rare include congestive heart failure, peptic ulcer perforation and other acute cardiovascular events and all of these will be clearly explained to the participants.

Justice – a clear criterion for inclusion and exclusion of patients. In other words, an absolute criterion for the selection process of patients. This study is a randomized controlled trial so all patients were equally allocated to receive the treatments. During participating in this study, all subjects were provided the standard lid hygiene and medications according to the standard guideline.

Expected or Anticipated Benefit Gain

This study aims to reveal the efficacy of bevacizumab in term of reduction level of lid margin telangiectasia and also improvement of MGD signs and symptoms. If we can prove the benefit of bevacizumab for MGD treatment so there are more options for MGD's patients. In the meantime, this study can point us which routes of drug

administration will provide the better effectiveness compare with the onset of action and invasiveness.

Risk and investigator's responsibility

- Risk: Intra-MG injection adverse events: from intra-MG bevacizumab injection study, there was no obvious adverse effect including local and systemic event. A mild hemorrhage was observed at the injection spot, which disappeared in the following day. No late-onset hemorrhage or infection occurred afterward.

Responsibility: Dot hemorrhage: compression at injected site, follow-up in the next day, advise for abnormal symptoms, Infection: perform operation in OR, aseptic technique, post-operative surveillance and antibiotics eye drop

- Risk: Topical bevacizumab eye drop adverse events: ocular discomfort, ocular pain, eye irritation, conjunctival redness, corneal epitheliopathy, corneal thinning, decrease corneal sensation, corneal infection, subconjunctival hemorrhage.[36]

Responsibility: Advise for abnormal symptoms and immediately stop the medication, non-preservative lubrications and gels, oral pain killers, advice patient to close their lids for 1 minute post application or to apply digital pressure on the puncta, silicone plugs could be place in the lower eyelids

- Risk: Systemic adverse events of bevacizumab, for example, hypertension, cardiovascular events, cerebrovascular events, CHF, GI perforation, RPLS, proteinuria, surgery and wound healing complications.

Responsibility: Acknowledgement of all side effects that may happen. Immediately stop the medication and advise patients to see the doctor again. Providing the standard care of patient according to the patient's conditions with multidisciplinary team.

Statistical analysis

Descriptive analysis was performed for baseline characteristics, which included sex, age, systemic comorbidities, ocular comorbidities, ophthalmic medications, non-ophthalmic medications, and clinical parameters. Demographic and baseline data will

be reported as percentage for categorical data and mean with standard deviation for continuous data. To analyze longitudinal data with uneven time points, generalized estimating equation (GEE) was used to compare data: OSDI score, telangiectasia grading, LMNA, corneal staining, MG quality, meiboscore, conjunctival redness, FBUT, first NIBUT, average NIBUT, LLT, and compliances. Fisher's exact test was used to compare nominal data – such as sex, systemic comorbidities, ocular comorbidities, ophthalmic medications, non-ophthalmic medications. Independent sample t test was used to compare continuous data – such as age. A probability of telangiectasia grading improvement by more than 1 was determined using the Kaplan-Meier method using log-rank testing. The relationship between telangiectasia grading and LMNA was analyzed with the analysis of variance (ANOVA). Statistical significance is p-value < 0.05 and statistical analyses were conducted using Stata Statistical Software (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Outcomes	Variables	Measurement	Data	Statistical test
Demographic data	Ex. sex, systemic comorbidities		Nominal	Fisher's exact test
	Ex. age		Continuous numerical	Independent sample t test
1° outcomes	Telangiectasia grading	0 - 3	Discrete numerical	GEE, Kaplan-Meier method using Log-rank test
	Lid margin neovascularized area (LMNA)	%	Continuous numerical	GEE
2° outcomes	OSDI	0 - 100	Discrete numerical	GEE
	MG quality	0 - 24	Discrete	GEE

			numerical	
	FBUT	Seconds Mean of 3 measurements	Continuous numerical	GEE
	Corneal staining	0 - 5	Discrete numerical	GEE
	Conjunctival redness	0 - 4	Discrete numerical	GEE
	LLT	Nanometers	Continuous numerical	GEE
	Meiboscore	0 - 6	Discrete numerical	GEE
	NIBUT	Seconds	Continuous numerical	GEE
Compliances	Frequency of standard lid hygiene/week	No./week	Discrete numerical	GEE
	Frequency of topical medication/day	No./day	Discrete numerical	GEE

Chapter 4 Results

We enrolled 31 patients for the treatment program. One patient was excluded from the study due to the severe MGD signs and symptoms, which required steroid eye drop for the treatment. No patient was lost to follow up, resulting in 15 patients per group ***(Figure 4)***. According to demographic data and clinical baseline characteristics, there

was no difference between the two groups except the shorter level of FBUT in the injection group *(Table 5)*.

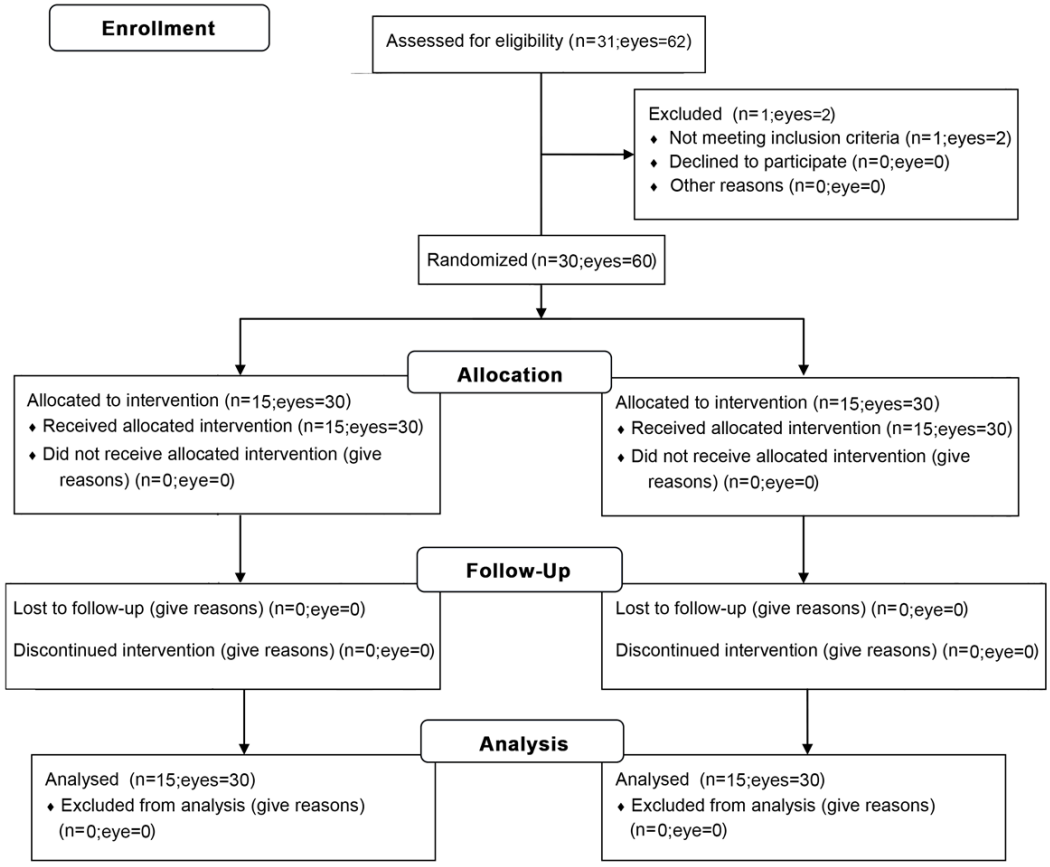


Figure 4 *CONSORT Flow Diagram*

Table 4 *Baseline Characteristics*

Variables	Injection group (n=15)	Eye drop group (n=15)
Female	11 (73.3%)	14 (93.3%)
Age (years), mean ± SD.	63.8 ± 8.14	63.4 ± 6.03
Systemic Comorbidities		
Hypertension	3 (20%)	7 (46.7%)

Dyslipidemia	2 (13.3%)	7 (46.7%)
Diabetic Mellitus	0 (0%)	2 (13.3%)
Others	5 (33.3%)	6 (40%)
Ocular Comorbidities		
Ocular Trauma	0 (0%)	0 (0%)
Ocular Surgery	2 (13.3%)	1 (6.7%)
Ocular Diseases	0 (0%)	1 (6.7%)
Ophthalmic Medications		
Artificial Tears	14 (93.3%)	14 (93.3%)
Lubricant Eye Gels	6 (40%)	5 (33.3%)
Non-Ophthalmic Medications		
Antihypertensive Medications	2 (13.3%)	8 (53.3%)
Antihyperlipidemic Medications	3 (20%)	9 (60%)
Antidepressants	3 (20%)	1 (6.7%)
Others	6 (40%)	8 (53.3%)
Patient-Reported Outcome		
OSDI Score (1-100), mean ± SD.	25.45 ± 14.28	23.73 ± 9.94
Primary Clinical Outcomes		
Telangiectasia Grading (0-3), mean ± SD.	2.23 ± 0.59	2.3 ± 0.53
Grade 1	3 (10%)	1 (3.3%)
Grade 2	15 (50%)	19 (63.3%)
Grade 3	12 (40%)	10 (33.3%)
Lid Margin Neovascular Area (%), mean ± SD.	4.6 ± 2.3	5.9 ± 3.2
Secondary Clinical Outcomes		
Corneal Staining (0-5), mean ± SD.	1.47 ± 1.27	0.87 ± 1.09
MG Quality (0-24), mean ± SD.	19.02 ± 3.82	18.79 ± 3.74
Meiboscore (0-6), mean ± SD.	2.21 ± 1.42	1.68 ± 1.11
Conjunctival Redness (0-4), mean ± SD.	0.77 ± 0.78	0.73 ± 0.7

FBUT (seconds), mean ± SD.	3.64 ± 1.52	4.88 ± 1.64
First-NIBUT (seconds)	7.28 ± 4.96	5.97 ± 3.41
Average-NIBUT (seconds)	9.69 ± 5.66	9.8 ± 4.42
LLT (nm), mean ± SD.	64 ± 26.24	72.33 ± 27.17

OSDI score

The OSDI score was considerably lowered from week 1 and remain stable until month 3 after the treatment in both groups ***(Table 5)***, ***(Figure 5)***. In the eye drop group, the OSDI was significantly decreased from 23.73 to 11.73 at week 1 (mean change -11.93, $p<0.001$) and persisted to month 3 ($p=0.234$). In the injection group, the OSDI was significantly improved from 25.45 to 18.18 at week 1 (mean change -7.27, $p<0.001$) and remain improve to month 3 ($p=0.213$). There is no significant difference between group at month 3 ($p=0.738$).

Primary outcomes

Telangiectasia Grading

The Kaplan-Meier survival analysis ***(Figure 6)*** show that in the injection group, a probability of telangiectasia grading improvement by more than 1 grade was 33.3% at week 1 and increased to 53.3% at month 3 post-treatment. In the eye drop group, the probability of telangiectasia grading improvement was 13.3% at month 2 and increased to 40% at month 3. However, there was no significant difference between 2 groups at month 3 ($p=0.126$). In the injection group, telangiectasia grading was substantially lowered from 2.23 to 2.05 at month 1 (mean change -0.26, $p=0.22$) and month 3 (mean change -0.56, $p<0.001$); whereas, in the eye drop group, telangiectasia grading was decreased significantly from 2.3 to 2.1 at month 2 ($p=0.024$) and significantly improved to month 3 ($p=0.015$) with no between-group difference in the decrease of telangiectasia grading at month 3 ($p=0.338$) ***(Table 6)***.

In telangiectasia grading subgroup analyses, both routes were found to improve telangiectasia grading significantly, however, the injection group was observed a faster reduction in telangiectasia grading compared with the eye drop group ***(Table 8)***.

Lid Margin Neovascularized Area (LMNA)

In the injection group, the percentage of LMNA was decreased from 4.6% to 4.2% at 1 week after the treatment (p=0.248) and remain stable until 2 months. At month 3, LMNA was significantly reduced to 3.8% (mean change -0.8%, p=0.005). In the eye drop group, the percentage of LMNA was decreased from 5.9 to 5.3 (mean change -0.7%, p=0.77) after month 3. The injection group possessed a greater decrease of LMNA than the eye drop group at month 3, otherwise; there is no significant difference between group (p=0.761) ***(Table 6)***.

The ANOVA was used to determine the relationship between telangiectasia grading values (1-3) and the mean values of LMNA, showed statistically significant differences between the mean values of LMNA in three telangiectasia grading scales of 1, 2, and 3 (p<0.001) ***(Table 10)***.

Secondary outcomes

Corneal Staining

In the injection group, corneal staining had decreased significantly from 1.47 to 0.83 at week 1 (mean change -0.68, p=0.001) whereas a significant decrease of corneal staining maintained at month 3 (p=0.344) **(Table 7)**. In the eye drop group, corneal staining was significantly reduced from 0.87 to 0.57 at month 3 (mean change -0.38, p=0.021). The improvement level of corneal staining in the injection group was significantly greater than the eye drop group at week 1, month 1, and month 2 (p<0.05); however, there was no between-group disparity at month 3 (p=0.675).

MG Quality

The MG quality score of the injection group had significantly improved from 19.02 to 16.21 at week 1 (mean change -2.81, p=0.001) and remained improve to month 3 after the treatment (p=0.127). The eye drop group had significant improvement of MG quality from 18.79 to 15.67 at month 1 (mean change -3.12, p=0.007) and maintained better to month 3 with significantly better MG quality, when compared with the injection group (p=0.021) ***(Table 7)***.

Meiboscore

In the injection group, there had been a significant improvement of meiboscore from 2.21 to 2 at week 1 (mean change -0.12, p=0.017) and significantly remained improve to month 3 after the treatment (p<0.001). In addition, in the eye drop group, there had been a significant decrease of meiboscore from 1.68 to 1.62 at month 1 (mean change -0.15, p=0.012) maintained improve to month 3 after the treatment (p=0.845). The decrease of meiboscore in the injection group was considerably greater than the eye drop group at month 2 (p=0.012) and month 3 (p<0.001) ***(Table 7)***.

Conjunctival Redness

In the injection group, conjunctival redness had significantly been reduced from 0.77 to 0.37 (mean change -0.47, p=0.001) at month 1 and persisted until month 3 after the treatment (p=0.089). However, in the eye drop group, a significant decrease of conjunctival redness was showed at month 3 after the treatment (p=0.017). No between-group difference in the decrease of conjunctival redness could be seen in each visit after the treatment (p>0.05) ***(Table 7)***.

Fluorescein Break Up Time

The value of FBUT in the injection group increased significantly from 3.64 to 4.96 seconds at month 2 (mean change 0.96, p=0.027) and remained stable at month 3 (p=0.667) ***(Table 7)***. However, such value in the eye drop group remained at the baseline in every visit (p>0.05). The FBUT in the injection group improved significantly at month 1 when compared with the eye drop group (p=0.019).

First NIBUT and Average NIBUT

There was no substantial difference from the baseline of both groups in every visit, and the between-group difference was insignificant at month 3 ***(Table 7)***.

Lipid Layer Thickness

There was no significant difference from the baseline of both groups in every visit, and the between-group difference was insignificant at month 3 ***(Table 7)***.

Adverse events

No systematic AE was detected in any patient. In the injection group, the most common symptom at post-operative day 1 is dot hemorrhage (16.7%), and there was no post-operative infection or active bleeding at the injection site at week 1 post treatment. Among eye drop patients, the most common AEs were eye irritation and transient eye redness, which were detected at 13.3% and 16.7% at month 1 and month 2, respectively. No local AE was observed at month 3 in both groups.

Compliances

Compliance of Lid Hygiene Care

There was significant difference between the two groups in terms of the frequency to perform lid hygiene ***(Table 9)***. At week 1, patients in the injection group performed lid hygiene less often than the eye drop group (4.53 vs. 6.33, p=0.042). The difference was much lessened at month 1 (p=0.651); however, it was widened at month 2, when the injection group having significantly greater frequency of lid hygiene performance than the eye drop group (6.43 vs. 5.3, p=0.047). At month 3, there was no significant difference between groups in performing lid hygiene (p=0.115).

No. of Tear Substitute/day

There had been no significant between-group difference in the use of artificial tears from week 1 to month 1. Nevertheless, the number of usages per day in the injection group was significantly greater than the eye drop group at month 2 (3.67 vs. 2.07, p=0.033) ***(Table 9)***.

No. of Bevacizumab Eye Drop/day

At month 3, patients in the eye drop group used bevacizumab 3.78 times per day on average.

Table 5 *Ocular Surface Disease Index (OSDI) Score*

OSDI Score (0-100)	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Baseline	25.45 ± 14.28	Reference	1	23.73 ± 9.94	Reference	1	Reference	1
1 week	18.18 ± 11.13	-7.27 (-11.11, -3.42)	<0.001*	11.73 ± 8.15	-11.93 (-16.15, -7.7)	<0.001*	4.66 (-1.06, 10.38)	0.11
1 months	16.06 ± 10.64	-9.39 (-13.24, -5.55)	<0.001*	12.04 ± 7.36	-11.47 (-15.69, -7.24)	<0.001*	2.07 (-3.65, 7.79)	0.478
2 months	15.82 ± 10.38	-10.04 (-13.89, -6.19)	<0.001*	14.46 ± 11.28	-9.12 (-13.35, -4.9)	<0.001*	-0.92 (-6.64, 4.8)	0.753
3 months	17.15 ± 10.15	-8.5 (-12.43, -4.57)	<0.001*	14.02 ± 14.24	-9.47 (-13.7, -5.25)	<0.001*	0.99 (-4.79, 6.77)	0.738

Patient-reported outcome

Table 6 *Primary Outcomes*

Telangiectasia Grading (0-3)	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Baseline	2.23 ± 0.59	Reference	1	2.3 ± 0.53	Reference	1	Reference	1
1 week	2.07 ± 0.68	-0.17 (-0.39, 0.06)	0.141	2.22 ± 0.66	-0.13 (-0.31, 0.04)	0.134	-0.03 (-0.31, 0.25)	0.817
1 months	2.05 ± 0.38	-0.26 (-0.48, -0.04)	0.022*	2.2 ± 0.62	-0.08 (-0.26, 0.09)	0.348	-0.18 (-0.46, 0.11)	0.223
2 months	2.17 ± 0.59	-0.08 (-0.31, 0.14)	0.461	2.1 ± 0.54	-0.2 (-0.37, -0.03)	0.024*	0.12 (-0.16, 0.4)	0.417
3 months	1.89 ± 0.86	-0.56 (-0.78, -0.33)	<0.001*	1.9 ± 0.54	-0.42 (-0.59, -0.24)	<0.001*	-0.14 (-0.42, 0.15)	0.338
Lid Margin Neovascularized Area (%)	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Baseline	4.6 ± 2.3	Reference	1	5.9 ± 3.2	Reference	1	Reference	1
1 week	4.2 ± 2.1	-0.3 (-0.9, 0.2)	0.248	6.4 ± 4.4	0.4 (-0.3, 1.2)	0.255	-0.8 (-1.7, 0.2)	0.108
1 months	4.2 ± 2.3	-0.4 (-0.9, 0.2)	0.208	6 ± 3.6	0 (-0.7, 0.8)	0.922	-0.4 (-1.3, 0.5)	0.396
2 months	4.1 ± 2.1	-0.5 (-1, 0.1)	0.105	5.9 ± 3.5	-0.1 (-0.8, 0.7)	0.858	-0.4 (-1.3, 0.5)	0.395
3 months	3.8 ± 2	-0.8 (-1.4, -0.2)	0.005*	5.3 ± 3	-0.7 (-1.4, 0.1)	0.077	-0.1 (-1.1, 0.8)	0.761

Clinician subjective and objective outcomes

Table 7 *Secondary Outcomes*

Variables	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Corneal Staining (0-5)								
Baseline	1.47 ± 1.27	Reference	1	0.87 ± 1.09	Reference	1	Reference	1
1 week	0.83 ± 0.86	-0.68 (-1.08, -0.28)	0.001*	0.83 ± 0.65	-0.17 (-0.49, 0.15)	0.306	-0.52 (-1.03, -0.01)	0.048*
1 months	0.93 ± 1.16	-0.63 (-1.03, -0.23)	0.002*	0.77 ± 0.78	0.02 (-0.3, 0.34)	0.918	-0.65 (-1.16, -0.14)	0.013*
2 months	0.57 ± 0.65	-0.83 (-1.23, -0.43)	<0.001*	0.97 ± 0.85	-0.05 (-0.37, 0.27)	0.759	-0.78 (-1.29, -0.27)	0.003*
3 months	1.14 ± 1.39	-0.49 (-0.89, -0.08)	0.02*	0.57 ± 0.59	-0.38 (-0.69, -0.06)	0.021*	-0.11 (-0.63, 0.41)	0.675
MG Quality (0-24)								
Baseline	19.02 ± 3.82	Reference	1	18.79 ± 3.74	Reference	1	Reference	1
1 week	16.21 ± 2.51	-2.81 (-4.47, -1.15)	0.001*	17.93 ± 3.58	-0.86 (-3.12, 1.4)	0.456	-1.95 (-4.75, 0.85)	0.173
1 months	16.68 ± 4.95	-2.34 (-4, -0.68)	0.006*	15.67 ± 6.61	-3.12 (-5.38, -0.87)	0.007*	0.79 (-2.02, 3.59)	0.583
2 months	16.93 ± 3.82	-2.09 (-3.75, -0.43)	0.014*	15.9 ± 5.72	-2.89 (-5.15, -0.64)	0.012*	0.81 (-2, 3.61)	0.573
3 months	17.43 ± 3.18	-1.49 (-3.18, 0.2)	0.084	13.95 ± 4.64	-4.84 (-7.09, -2.58)	<0.001*	3.34 (0.51, 6.17)	0.021*

Variables	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Meiboscore (0-6)								
Baseline	2.21 ± 1.42	Reference	1	1.68 ± 1.11	Reference	1	Reference	1
1 week	2 ± 1.31	-0.12 (-0.21, -0.02)	0.017*	1.63 ± 1.15	-0.05 (-0.17, 0.07)	0.404	-0.07 (-0.22, 0.09)	0.404
1 months	1.87 ± 1.25	-0.24 (-0.33, -0.15)	<0.001*	1.62 ± 1.16	-0.15 (-0.27, -0.03)	0.012*	-0.09 (-0.24, 0.06)	0.254
2 months	1.75 ± 1.24	-0.35 (-0.45, -0.26)	<0.001*	1.56 ± 1.17	-0.15 (-0.27, -0.04)	0.01*	-0.2 (-0.35, -0.04)	0.012*
3 months	1.59 ± 1.28	-0.5 (-0.6, -0.41)	<0.001*	1.57 ± 1.18	-0.14 (-0.26, -0.02)	0.021*	-0.37 (-0.52, -0.21)	<0.001*
Conjunctival Redness (0-4)								
Baseline	0.77 ± 0.78	Reference	1	0.73 ± 0.7	Reference	1	Reference	1
1 week	0.6 ± 0.63	-0.22 (-0.48, 0.05)	0.107	0.57 ± 0.42	-0.17 (-0.43, 0.09)	0.208	-0.05 (-0.42, 0.32)	0.791
1 months	0.37 ± 0.58	-0.47 (-0.73, -0.2)	0.001*	0.63 ± 0.69	-0.1 (-0.36, 0.16)	0.45	-0.37 (-0.74, 0)	0.052
2 months	0.6 ± 0.81	-0.3 (-0.56, -0.04)	0.026*	0.5 ± 0.82	-0.23 (-0.49, 0.03)	0.078	-0.07 (-0.44, 0.3)	0.724
3 months	0.39 ± 0.66	-0.49 (-0.75, -0.22)	<0.001*	0.4 ± 0.71	-0.32 (-0.58, -0.06)	0.017*	-0.17 (-0.54, 0.2)	0.375

Variables	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
FBUT (seconds)								
Baseline	3.64 ± 1.52	Reference	1	4.88 ± 1.64	Reference	1	Reference	1
1 week	4.8 ± 1.57	0.66 (-0.18, 1.51)	0.124	4.82 ± 2.01	0.05 (-0.56, 0.66)	0.873	0.62 (-0.43, 1.66)	0.247
1 months	4.67 ± 2.04	0.81 (-0.04, 1.66)	0.061	4.31 ± 0.93	-0.44 (-1.05, 0.17)	0.156	1.25 (0.21, 2.29)	0.019*
2 months	4.96 ± 1.91	0.96 (0.11, 1.8)	0.027*	4.64 ± 1.39	0.01 (-0.6, 0.62)	0.974	0.95 (-0.1, 1.99)	0.075
3 months	5.12 ± 2.21	0.77 (-0.1, 1.63)	0.082	4.29 ± 1.97	-0.11 (-0.72, 0.5)	0.73	0.89 (-0.16, 1.94)	0.098
First NIBUT (seconds)								
Baseline	7.28 ± 4.96	Reference	1	5.97 ± 3.41	Reference	1	Reference	1
1 week	6.23 ± 4.61	-0.76 (-2.68, 1.16)	0.439	9.45 ± 6.93	1.57 (-0.61, 3.74)	0.157	-2.33 (-5.23, 0.58)	0.116
1 months	7.28 ± 3.22	-0.46 (-2.38, 1.47)	0.643	7.76 ± 3.83	0.18 (-1.99, 2.35)	0.87	-0.64 (-3.54, 2.27)	0.667
2 months	5.22 ± 2.97	-1.85 (-3.77, 0.07)	0.06	6.22 ± 5.77	-0.05 (-2.22, 2.13)	0.967	-1.8 (-4.7, 1.1)	0.224
3 months	6.84 ± 5.89	-0.61 (-2.57, 1.35)	0.544	6.49 ± 5.69	-0.38 (-2.55, 1.79)	0.731	-0.23 (-3.16, 2.7)	0.877

Variables	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Average NIBUT (seconds)								
Baseline	9.69 ± 5.66	Reference	1	9.8 ± 4.42	Reference	1	Reference	1
1 week	9.09 ± 5.47	0.19 (-1.71, 2.1)	0.843	10.74 ± 6.39	-1.11 (-3.31, 1.09)	0.324	1.3 (-1.61, 4.21)	0.382
1 months	8.89 ± 3.71	-0.51 (-2.42, 1.39)	0.599	11.23 ± 3.88	0.27 (-1.93, 2.47)	0.811	-0.78 (-3.69, 2.13)	0.6
2 months	8.2 ± 3.36	-1.14 (-3.04, 0.77)	0.243	9.43 ± 5.8	-0.64 (-2.84, 1.56)	0.569	-0.5 (-3.41, 2.42)	0.739
3 months	9.09 ± 6.12	0.24 (-1.71, 2.18)	0.811	9.96 ± 6.63	-1.31 (-3.51, 0.89)	0.243	1.54 (-1.4, 4.48)	0.305
LLT (nm)								
Baseline	64 ± 26.24	Reference	1	72.33 ± 27.17	Reference	1	Reference	1
1 week	62.33 ± 31.38	-2.63 (-14.16, 8.89)	0.654	69.67 ± 27.24	0.43 (-9.69, 10.55)	0.933	-3.07 (-18.4, 12.27)	0.695
1 months	60.6 ± 30.96	-4.07 (-15.59, 7.46)	0.489	59.27 ± 26.66	-3.4 (-13.52, 6.72)	0.51	-0.67 (-16, 14.67)	0.932
2 months	56.07 ± 34.66	-4.4 (-15.93, 7.13)	0.454	65.33 ± 27.49	-0.07 (-10.19, 10.05)	0.99	-4.33 (-19.67, 11)	0.58
3 months	56.14 ± 32.9	-8.51 (-20.28, 3.25)	0.156	56.67 ± 27.92	-9.13 (-19.25, 0.99)	0.077	0.64 (-14.86, 16.13)	0.936

Clinician subjective and objective outcomes

Table 8 *Subgroup Analysis of Telangiectasia*

Telangiectasia Grade 2	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Telangiectasia Grading (0-3)	N=15			N=19				
Baseline	2 ± 0	Reference	1	2 ± 0	Reference	1	Reference	1
1 week	1.83 ± 0.62	-0.17 (-0.4, 0.06)	0.156	1.92 ± 0.5	-0.08 (-0.29, 0.13)	0.46	-0.09 (-0.4, 0.23)	0.583
1 months	1.95 ± 0.3	-0.05 (-0.28, 0.18)	0.671	1.95 ± 0.33	-0.05 (-0.26, 0.16)	0.622	0 (-0.31, 0.32)	0.987
2 months	2.27 ± 0.46	0.27 (0.04, 0.5)	0.023*	1.84 ± 0.41	-0.16 (-0.37, 0.05)	0.14	0.42 (0.11, 0.74)	0.008*
3 months	1.67 ± 0.66	-0.34 (-0.58, -0.1)	0.006*	1.66 ± 0.41	-0.34 (-0.55, -0.13)	0.001*	0.01 (-0.31, 0.33)	0.964
Lid Margin Neovascularized Area (%)	N=7			N=10				
Baseline	3.9 ± 1.7	Reference	1	4.4 ± 2.3	Reference	1	Reference	1
1 week	3.9 ± 2.3	0 (-0.8, 0.8)	0.972	4.3 ± 2	-0.1 (-0.6, 0.4)	0.662	0.1 (-0.8, 1)	0.781
1 months	3.7 ± 1.9	-0.2 (-1, 0.6)	0.588	4.2 ± 1.7	-0.3 (-0.8, 0.2)	0.31	0 (-0.9, 1)	0.922
2 months	3.7 ± 1.4	-0.2 (-1, 0.6)	0.591	4.2 ± 1.9	-0.2 (-0.7, 0.3)	0.39	0 (-0.9, 0.9)	0.99
3 months	3.4 ± 2	-0.5 (-1.3, 0.3)	0.194	3.8 ± 1.8	-0.6 (-1.2, -0.1)	0.015*	0.1 (-0.8, 1)	0.815

Telangiectasia Grade 3	Injection group			Eye drop group			Between treatment	
	Mean ± SD.	Mean change (95%CI)	p-value	Mean ± SD.	Mean change (95%CI)	p-value	Mean difference (95%CI)	p-value
Telangiectasia Grading (0-3)	N=12			N=10				
Baseline	3 ± 0	Reference	1	3 ± 0	Reference	1	Reference	1
1 week	2.46 ± 0.5	-0.54 (-0.88, -0.2)	0.002*	2.7 ± 0.67	-0.3 (-0.61, 0.01)	0.056	-0.24 (-0.71, 0.22)	0.308
1 months	2.08 ± 0.56	-0.92 (-1.26, -0.58)	<0.001*	2.8 ± 0.42	-0.2 (-0.51, 0.11)	0.202	-0.72 (-1.18, -0.25)	0.003*
2 months	2.25 ± 0.45	-0.75 (-1.09, -0.41)	<0.001*	2.6 ± 0.52	-0.4 (-0.71, -0.09)	0.011*	-0.35 (-0.82, 0.12)	0.14
3 months	2.04 ± 0.58	-0.96 (-1.3, -0.62)	<0.001*	2.3 ± 0.48	-0.7 (-1.01, -0.39)	<0.001*	-0.26 (-0.72, 0.21)	0.276
Lid Margin Neovascularized Area (%)	N=7			N=5				
Baseline	5.8 ± 2.3	Reference	1	9 ± 2.5	Reference	1	Reference	1
1 week	5.1 ± 1.5	-0.7 (-1.6, 0.2)	0.106	10.5 ± 5.1	1.5 (-0.3, 3.3)	0.103	-2.2(-4.1, -0.4)	0.018*
1 months	5.2 ± 2.3	-0.6 (-1.5, 0.3)	0.191	9.6 ± 3.7	0.6 (-1.2, 2.5)	0.488	-1.2 (-3.1, 0.6)	0.193
2 months	5 ± 2.2	-0.8 (-1.7, 0.1)	0.071	9.2 ± 3.7	0.3 (-1.6, 2.1)	0.788	-1.1 (-2.9, 0.8)	0.261
3 months	4.6 ± 1.7	-1.2(-2.1, -0.3)	0.009*	8.3 ± 2.8	-0.7 (-2.5, 1.1)	0.443	-0.5 (-2.3, 1.4)	0.618

Table 9 *Compliance for Lid Hygiene Care and Use of Tear Substitutes*

Variables	Injection group (n=15)	Eye drop group (n=15)	p-value
Lid Hygiene Care (day/week)			
1 week	4.53 ± 3.04	6.33 ± 1.19	0.042*
1 months	5.93 ± 1.83	6.2 ± 1.32	0.651
2 months	6.43 ± 0.86	5.3 ± 1.93	0.047*
3 months	6.29 ± 0.91	5.37 ± 1.91	0.115
Use of Tear Substitutes (drop/day)			
1 week	4.33 ± 2.41	3.23 ± 2.65	0.244
1 months	3.27 ± 2.46	2.37 ± 2	0.281
2 months	3.67 ± 2.01	2.07 ± 1.88	0.033*
3 months	3.61 ± 2.11	2.57 ± 1.84	0.168

Table 10 *Analysis of Variance (ANOVA)*

Telangiectasia Grading	Lid Margin Neovascularized Area (%) Mean ± SD.			
	N.	Injection group	N.	Eye drop group
1	1	0.76	0	-
2	7	3.92 ± 1.66	10	3.66 ± 2.37
3	7	5.80 ± 2.26	5	8.97 ± 2.53

Figure 5 *Mean Difference of OSDI Score*

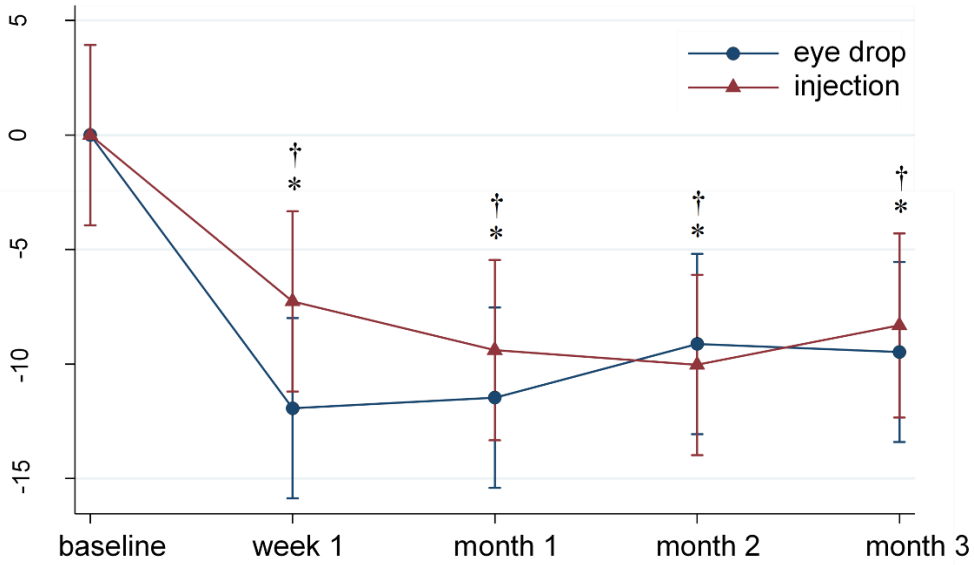
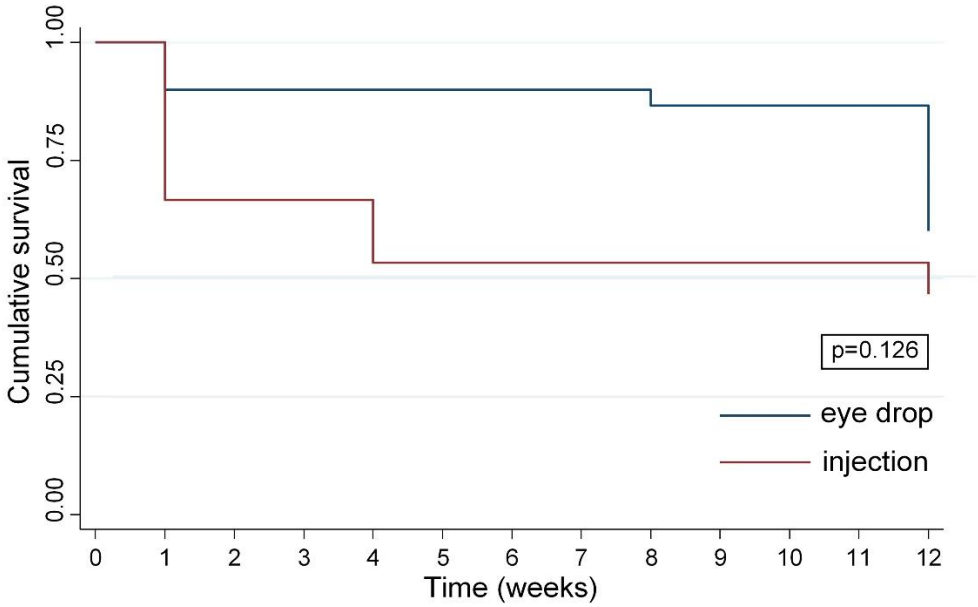


Figure 5 Mean (± SD) difference from baseline of Ocular Surface Disease Index (OSDI) score at each visit. †P < 0.05, within-eye drop group differences. *P < 0.05, within-injection group differences.

Figure 6 *Kaplan-Meier Survival Analysis of Telangiectasia Grading Improvement*



No. at risk

Eye drop	30	27	27	26	18
Injection	30	20	16	16	14

Figure 7 Mean Difference of Lid Margin Neovascularized Area (LMNA)

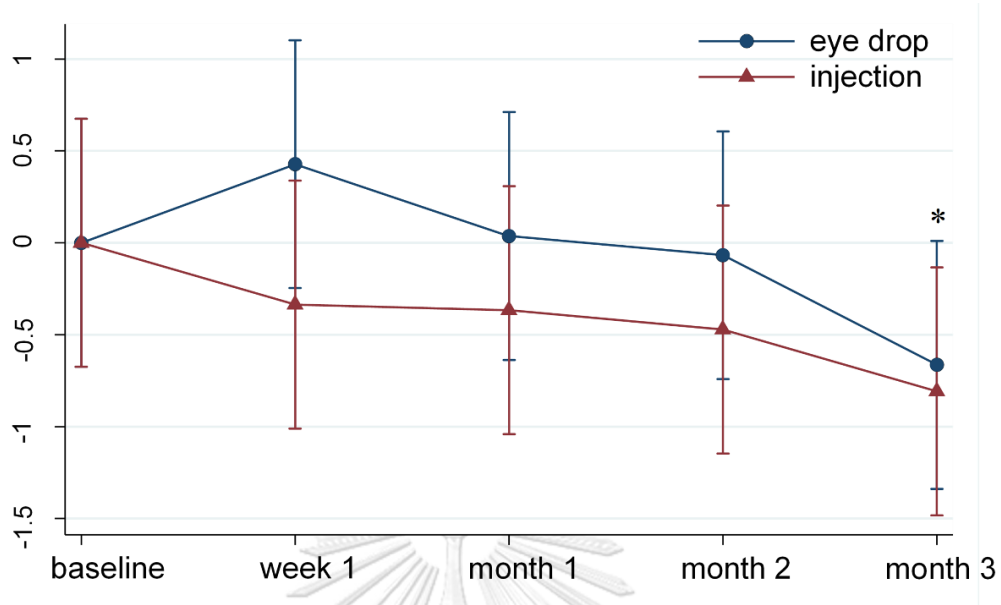


Figure 7 Mean (± SD) difference from baseline of Lid Margin Neovascularized Area (LMNA) at each visit. *P < 0.05, within-injection group differences.

Chapter 5 Discussion and Conclusions

This study was the first open-label observer blinded randomized controlled trial, which observed the effect of bevacizumab in the form of eye drop and intra-MG injection on the reduction of lid margin telangiectasia and improvement of MGD signs and symptoms. The primary outcomes were telangiectasia grading and LMNA. This study showed that single 2.5% intra-MG injection of bevacizumab with regular lid hygiene could significantly reduce telangiectasia grading and LMNA, while the bevacizumab eye drop could significantly decrease only telangiectasia grading after 3 months. The injection group showed improvement of corneal staining, meiboscore, and FBUT when compared with the eye drop group. However, both groups showed significant improvement of dry eye symptoms and MGD signs include corneal staining, MG quality, meiboscore, and conjunctival redness.

The study results align with Jiang's research in 2018, which involved the use of single 2.5% bevacizumab intra-MG injection. In Jiang's study, lid vascularity, OSDI score, MG expressivity, MG quality, conjunctival redness, corneal staining, and FBUT in patients were significantly improved at week 1 and sustained to month 3. On the other hand, Kasetsuwan et al. reported a significant improvement of OSDI score, corneal staining, and FBUT in DED patients treated with 0.05% bevacizumab eye drop at month 3 compared with the control group.

For the primary outcomes, telangiectasia grading in the injection group started to decrease significantly from month 1 after treatment and continued to lower until month 3. Such trend in the eye drop group can be noticed from month 2 after the instillation. However, there was no significant between-group difference at month 3. In this study, the values of LMNA were obtained by the analysis of slit lamp photography performed with image analysis software (Cell Sens Dimension software®), which is considered an objective measurement. However, this study found that change in LMNA values observed in patients who used eye drops was inconsistent with the grade of telangiectasia, which is a widely accepted qualitative method for clinical outcome measurement. A previous study[33] showed that telangiectasia grading of the upper

eyelids performed by general ophthalmology only had moderate reliability (0.59). On the contrary, our data showed that there was a significant correlation between the grade of telangiectasia and LMNA in each of the telangiectasia grading groups ($p<0.001$). Therefore, in our view, LMNA could also be used as a computer-assisted quantitative measurement for diagnosis and post-treatment monitoring of patients. Nevertheless, this parameter may not be sensitive to change especially in eye drop group. We assumed ethnicity or skin color might be an obstacle to detect changes in blood vessels other than the between-visit position of eyelid.

The OSDI score of both groups had reduced significantly from week 1 to month 3 with no between-group difference. According to the study results of subconjunctival bevacizumab injection in DED[31] (Jiang et al. 2015), intra-MG bevacizumab injection in MGD[23] (Jiang et al. 2018), and 0.05% bevacizumab eye drop in DED[25] (Kasetsuwan et al. 2020), bevacizumab can significantly improve dry eye symptoms. The decrease in corneal staining and lid margin inflammation and improvement of tear film instability can account for the improvement of OSDI score in MGD. In addition, VEGF and VEGFR2 are related to the pathogenesis of neuropathic pain. According to Lin et al.,[37] injection of anti-VEGF in neuropathic pain model in rats can alleviate chronic neuropathic pain by reducing the expressions of VEGFR2. Bevacizumab treatment can reduce dry eye symptoms as early as week 1, compared with other anti-inflammatory medications, and the mean OSDI score of less than 13[38] was reported at week 1 and month 1 in the eye drop group. However, the earliest time that the 5% lifitegrast can improve eye dryness score is week 2.[39,40] Similarly, the duration for cyclosporine eye drop (CsA) to improve dry eye symptoms ranges from 1 month[41] to 3 month.[42]

The MG quality of the injection group had significantly improved its maximum level at week 1 and gradually lowered until month 3. However, in the eye drop group, the increasingly significant improvement of MG quality could be observed from month 1 to month 3 with better improvement than the first group at month 3. This is in line with the trend of meiboscore. Choi et al. and Liu et al.[43,44] observed that after subjects underwent intense pulsed light (IPL) treatments at month 1, there was a significant reduction in tear

cytokine levels (IL-6, IL-10, IL-17A and TNF-α). This reduction in multiple cytokines positively correlated with the improvement in MG quality and expressibility, which was consistent with meiboscore changes. Ban et al.[45] discovered a positive correlation between MG expression of secretion and meiboscore (R=0.404, p=0.016). Furthermore, Arita et al.[46] explained that dark lesions observed in noncontact meibography could be attributed to degenerative meibum aside from MG dropout. Therefore, reducing inflammation from bevacizumab treatment could result in improvements in MG quality, meiboscore, as well as improvement of the integrity of tear film lipid layer[43] (FBUT), as shown in ***Figure 8***.

In injection group, there was a decrease in conjunctival redness started from month 1, whereas, in the eye drop group, the significant decrease started from month 2 after the treatment. The reduction in conjunctival redness was consistent with telangiectasia grading ***(Figure 8)***. Furthermore, In telangiectasia subgroup analyses, both routes were found to improve telangiectasia grading, however, we observed a faster reduction of telangiectasia grading in the injection group. This could be explained by multiple anti-VEGF mechanisms that could reduce angiogenesis, vascular permeability, lymphangiogenesis, and the infiltration of inflammatory cells, which leads to improvements in lid margin and ocular surface inflammation. The change in size of blood vessels and areas of neovascularization might require a longer period of time, depending on the route of administration, drug concentration, and drug penetration (intact epithelium and large molecular weight of bevacizumab (149 kDa)).[47,48] These factors could explain why bevacizumab eye drop is as effective as injection route but has slower response. However, the eye drop route is non-invasive and is suitable to be used as routine treatment.

In the injection group, corneal staining inclined to lower significantly from week 1 to month 3, but, in the eye drop group, there was significant improvement at month 3 post treatment. However, the duration of staining reduction is shorter than that of other anti-inflammatory medications. For instance, 5% lifitegrast can improve inferior corneal staining score at month 3,[49] while CsA can reduce corneal staining from month 1[50] to

month 4.[42] Corneal staining[41] is caused by the insult to corneal and conjunctival epithelium. The reduction of inflammation, together with the improvement of tear film stability, not only helps rehabilitate ocular surface health but also improves nerve ending, which can further enhance neurosensory functions. Moreover, the bevacizumab treatment in vivo could promote the regeneration of corneal sensory nerves[51] after the presence of herpes simplex virus type 1 stromal keratitis in a mouse model.

In this study, the OSDI score and MGD signs were improved. Nevertheless, the results of some objective tests (i.e. NIBUT, LLT) did not changed accordingly. According to the systematic review[52] in 2015, Bartlett et al. revealed that signs and symptoms of DED had low to moderate correlation and inconsistency in the perspective of diagnosis and treatment monitoring of DED. There is a significantly moderate correlation between NIBUT and dry eye symptoms[53] and the result of NIBUT is more dependable than the result of FBUT.[54] Besides, Cox et al.[55] in 2015 reported poor agreement of between-visit repeatability of NIBUT.

The LLT cut off value[56] for screening obstructive MGD (≤ 75 nm) had the sensitivity of 65.8% and specificity of 63.4%. Moreover, LLT is affected by age, sex, and other factors. In our study, mean of LLT of both groups aligned with the previous studies.[57,58] After the treatment, there was no significant change in LLT of both groups at month 3. Consistently with recently published trials[43,59] that reported no change in LLT after IPL therapy at week 12 to week 15, respectively. On the other hand, changes in LLT may require a longer period of monitoring. According to Arita et al.,[60] significant improvement of LLT after IPL treatment can be observed at month 6.

In 2009, Bock et al.[61] found that the application of 0.5% bevacizumab eye drop over the course of 2 weeks had no significant side effect on corneal epithelial wound healing after corneal injury, corneal morphology, and corneal nerve density in normal murine cornea. In this study, the 0.05% bevacizumab eye drop can cause 13.3 % of ocular irritation at month 1 and 6.6 % of transient eye redness at month 2 after treatment. No local AE of both routes was observed at month 3. However, CsA can cause up to

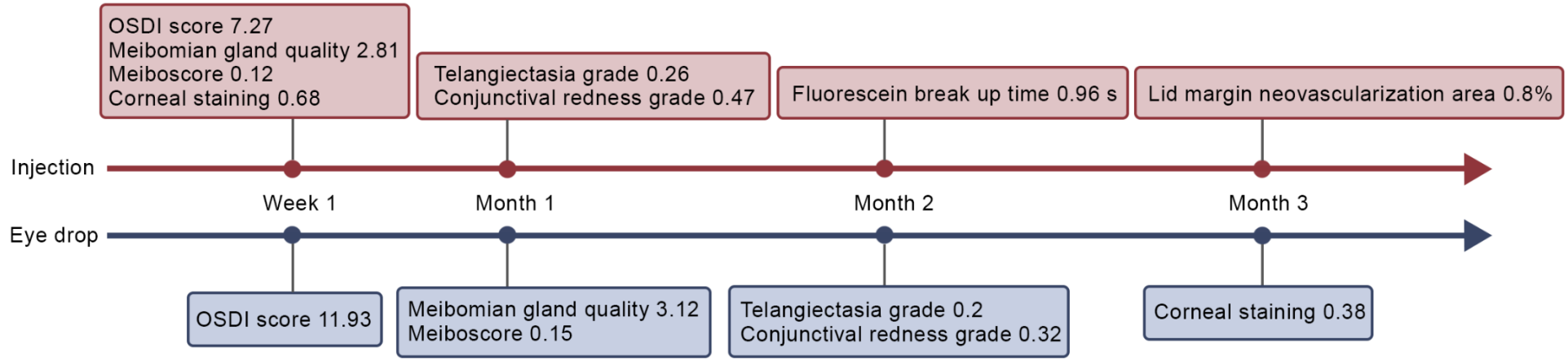
29 % burning[62] at instillation site, and 16.2% of dysgeusia[39] can be detected upon using 5% lifitegrast.

We suggest intra-MG injections as a suitable treatment method for MGD patients with moderate to severe lid margin telangiectasia or poor compliance for topical eye drops. However, those MGD patients who refused to be treated with injection could eventually benefit from bevacizumab eye drops after 3 months of treatment. For post-trial drug assessment, bevacizumab is currently available at King Chulalongkorn Memorial Hospital but this drug is non-essential drug (NED). We estimated the cost per unit which cannot be reimbursed in Thailand, depending on the route of drug administration. Bevacizumab eye drops cost approximately 250 baht per month and single intra-MG bevacizumab injection costs approximately 350 baht. Besides, LMNA parameter and Anticipated Significant Improvement of Eight Clinical Outcomes ***(Figure 8)*** could be used to monitor response of MGD patients after bevacizumab treatment. Nevertheless, a limitation of this study is the lack of a vehicle control group for scientific and ethical issues. In the future, more randomized studies are needed to determine the suitable concentration, frequency, duration of treatment course, number and duration to repeat intra-MG injection. The scope of this study does not cover a before-and-after treatment comparison between the inflammatory cytokines and nerve fiber density.

Conclusions

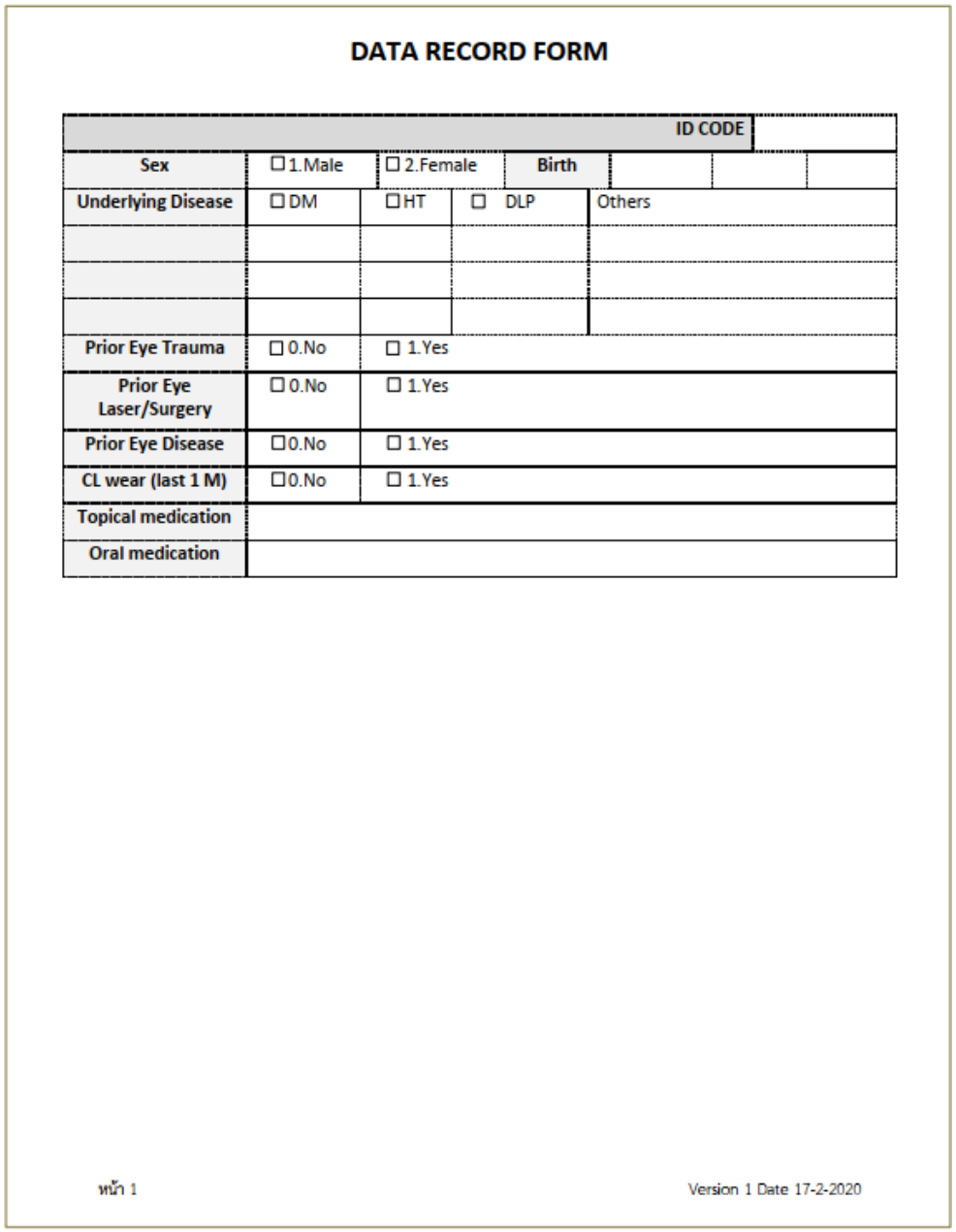
Both routes of intra-MG injection and eye drop bevacizumab administrations were safe and effective in reducing lid margin telangiectasia and signs and symptoms of MGD. Therefore, both routes of administration could be an alternative or adjunctive treatment with the standard lid hygiene for MGD patients.

Figure 8 *Anticipated Significant Improvement of Eight Clinical Outcomes*



Supplements

Figure 9 *Case Record Form*



DATA RECORD FORM

				ID CODE		
Sex	☐1.Male	☐2.Female	**Birth**			
Underlying Disease	☐DM	☐HT	☐ DLP	Others		
Prior Eye Trauma	☐0.No	☐ 1.Yes				
Prior Eye Laser/Surgery	☐0.No	☐ 1.Yes				
Prior Eye Disease	☐0.No	☐ 1.Yes				
CL wear (last 1 M)	☐0.No	☐ 1.Yes				
Topical medication						
Oral medication						

หน้า 1

Version 1 Date 17-2-2020

Figure 10 *QR Code of Standard Lid Hygiene*

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VITA

NAME	Chitchanok Tantipat
DATE OF BIRTH	5 February 1988
PLACE OF BIRTH	Chanthaburi province
INSTITUTIONS ATTENDED	Faculty of medicine, Chulalongkorn University
HOME ADDRESS	284 Sukhunvit 65 Road, North Phrakhanong sub-district, Wattana district, Bangkok 10110 Thailand
PUBLICATION	The efficacy between conventional lid hygiene and additional thermal pulsatile system in meibomian gland dysfunction patients treated with long-term anti-glaucoma medications in a randomized controlled trial.