Effectiveness of Low Level Light Therapy and Intense Pulse Light on Mite Count as Adjuntive Therapies in Demodex Blepharitis Using Artificial Intelligent Program (Ai-Demodex): A Factorial Randomized Sham-Controlled Trial

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Full Proposal

1. Proposal Title

English : Effectiveness of Low Level Light Therapy and Intense Pulse Light on Mite Count as Adjuntive Therapies in Demodex Blepharitis Using Artificial Intelligent Program (Ai-Demodex): A Factorial Randomized Sham-Controlled Trial

2. Investigators

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3. Rationale

Demodex blepharitis is a prevalent ocular condition arising from an overgrowth of Demodex mites, part of the normal ocular flora, on the eyelids. Although these mites typically reside on the skin, an increased population can lead to inflammation and eyelid infection. A robust correlation exists between demodex blepharitis and meibomian gland dysfunction (MGD) which is the primary cause of evaporative dry eye.

Kasetsuwan et al. in 2017 from KCMH,Thailand. The prevalence of ocular Demodex blepharitis in Thailand, detected by microscopic examination, was 42% (Demodex folliculorum). Among patients with ocular Demodex infestation, 69% had cylindrical dandruff. A higher detection rate of 79% was achieved using semi-nested PCR.(1)

Flores de Venecia et al. documented that demodex blepharitis has strong correlation with MGD. An 85% incidence of Demodex in MGD patients compared to 34% in healthy controls (without lid margin disease) (2). The severity of Demodex infestation aligns with the extent of MG damage, indicating a direct association(3).

Historically, demodex blepharitis and MGD were treated together using mechanical treatment like warm compression and lid scrubbing and pharmacological treatment as Tea Tree Oil (TTO)(4). An FDA-approved solution for the disease is lotilaner ophthalmic solution, 0.25% (5), but its high cost at 1,957 USD per 10 ml is a notable drawback.

Hyun et al. (2012) found that only 7% of patients were Demodex-free after eyelid scrubs without the use of TTO. Therefore, in practice, we now recommend that patients with Demodex blepharitis treat their condition with eyelid scrubs containing TTO for higher treatment efficacy.(6)

Patients often find eyelid hygiene tedious and time-consuming, leading to poor treatment compliance (7). Recent innovations in in-office MGD management include Intense Pulsed Light (IPL), proposed as an adjunctive therapy for demodicosis. IPL's heat transfer may kill Demodex mites and soften meibum, reducing inflammatory mediators. Limitations include cost and inapplicability to patients with darker skin tones (Fitzpatrick V or VI), light-intolerant individuals, or pediatric patients (4).

An alternative cellular-level therapy overcoming IPL limitations is Low-Level Light Therapy (LLLT), using near-infrared light to induce mitochondrial light absorption and cell photoactivation, causing changes in inflammatory protein expression (8). This technology, recognized by NASA for

plant growth and astronaut wound healing (9), has diverse applications, including dermatology for Androgenetic Alopecia (AGA), skin rejuvenation, body contouring (10), and orthopedics for reducing edema, inflammation, and relieving pain and stiffness.

For direct effect to demodex mite Avci et al. (2013) and Sulek et al found that blue light activates porphyrins, leading to an antibacterial effect(11, 12). Rhee et al. (2023) conducted a comprehensive review revealing that Demodex mites can carry Streptococcus and Staphylococcus species on their surface, while Bacillus oleronius is present in their abdomen.(4)

Recently, LLLT has been used for MGD, with devices allowing combined (13) or separate (14) treatment with IPL or LLLT emerging as alternatives for MGD management.

Evaporative dry eye in dry eye disease (DED) is a long-term, multifactorial condition affecting the tears and the ocular surface, with a huge worldwide impact. It encompasses two main types: aqueous deficient and evaporative dry eye(15). In the USA, DED incurs an indirect annual cost of US $73.12 billion, equating to US $15,596 per person, due to diminished productivity(16). Treatment costs vary by DED severity, ranging from US $300 to US $1100 per person across different countries (17). The global DED market, valued at USD 5.22 billion in 2019, is anticipated to reach USD 6.54 billion by 2027, with an annual growth rate of 4.7%.

Beyond financial concerns, various studies highlight DED as a burdensome condition associated with depression, which is a hidden problem often overlooked.

Artificial intelligent technology has revolutionized the field of ophthalmology, particularly in the analysis of meibomian gland images. By incorporating AI-based meibography, we can improve the accuracy and consistency of assessments related to glandular health, a crucial factor in managing MGD associated with Demodex blepharitis. Yuan et al. (2021) have demonstrated a reliable AI-based methodology for normalizing and segmenting meibomian gland images, offering substantial advancements over conventional techniques(18)

Our study pioneers a Factorial Randomized Control Trial (RCT) investigating Low-Level Laser Therapy (LLLT) for the treatment of demodex blepharitis, comparing it with Intense Pulsed Light (IPL), IPL combined with LLLT, and mechanical with pharmacological approaches. The aim is to address the limitations of mechanical with pharmacological treatment for demodex blepharitis, particularly in terms of the efficacy of innovative laser treatments, improved compliance, and reduced disease burden, ultimately leading to better treatment outcomes and

used AI-based meibography to evaluate the meibography photos which taken from demodex blepharitis patient before and after treatment.

Novel questions

1.Will Low-Level Light Therapy (LLLT) and/or Intense Pulsed Light (IPL) therapy synergistically or antagonistically improve the mean reduction of Demodex mite count in patients with Demodex blepharitis?

2.Will Low-Level Light Therapy (LLLT) and/or Intense Pulsed Light (IPL) therapy synergistically or antagonistically improve the Meibomian Gland Dysfunction (MGD) stage in patients with Demodex blepharitis?

4. Literature Review

4.1) Treatment Parameters for Demodex count : Primary outcome

No previous study has reported the count of demodex mites through Low-Level Light Therapy (LLLT). It has been mentioned that Intense Pulsed Light (IPL) improves ocular surface parameters, while studies on the treatment parameters of demodex blepharitis have predominantly focused on the following outcomes:

Advantages of Using Demodex Count as a Primary Outcome

Objective Outcome: The Demodex count provides a quantifiable and objective measurement, reducing the potential for subjective bias in assessing treatment efficacy.

Correlation with Recurrence: A high Demodex count has been shown to correlate with the recurrence of a series of refractory ocular surface diseases, indicating its potential as a predictive marker for disease management and long-term outcomes.(19)

Disadvantages of Using Demodex Count as a Primary Outcome

Poor Correlation with Symptoms: The Demodex count may not correlate well with the severity of symptoms experienced by patients. As a result, a reduction in Demodex count may not necessarily translate to symptomatic relief, potentially limiting its effectiveness as a standalone primary outcome measure. (AAO , (20))

4.2) Meibomian Gland Dysfunction Treatment Parameter: Secondary outcome

Regional Demodex blepharitis is strongly associated with the severity of meibomian gland dysfunction. For instance, Hyun Koo et al. in 2012 demonstrated that TTO treatment could improve both Demodex count and OSDI score(6), while Zhang et al. in 2019 showed a significant statistical improvement in TBUT.(21)

Only one preliminary study has suggested that LLLT, with four weekly sessions lasting 15 minutes each and combined with non-preservative tear application, might improve tear meniscus height.

There is currently no research on the effects of blue light LLLT on ocular surface parameters in Meibomian Gland Dysfunction. Therefore, if our study is successful, it will provide valuable insights into this knowledge gap.

4.3) Epilation strategies

The lashed epilation technique lacks a standardized method. Numerous studies conducted from 1967 to 2024 have demonstrated that approximately 40% of these studies utilize the Modified Coston method as outlined by Gao et al. in 2005. In this method, two lashes containing cylindrical dandruff (CD) were extracted from each eyelid of every participant using fine forceps. These lashes were then individually placed at opposite ends of a glass slide for observation under a slit-lamp biomicroscope (SL220; Carl Zeiss, Oberkochen, Germany) at a magnification of ×25. This process resulted in eight lashes (4 per each eye) being prepared across four slides for each participant. A coverslip was applied to each lash, followed by the slow pipetting of 20 μL of saline at the coverslip's edge until it surrounded the lash. Subsequently, 20 μL of 100% alcohol (Sigma-Aldrich, St. Louis, MO) was pipetted at the same edge, extending the counting period for up to 20 minutes to facilitate the migration of embedded Demodex from the CD. The Demodex count was performed thrice under the microscope, and all samples were photographed using standard procedures by the same specialist. The presence of Demodex in at least one of the eight eyelashes was considered Demodex-positive.(22)

Regarding epilation techniques, there are three primary methods:

A) Direct pulling,

B) Rotating the lash before epilation, and

C) Removing the cylindrical dandruff from the base of the lash before epilation.

Bitton et al. in 2024 concluded that Technique B, involving rotating the lash before epilation, yielded the highest Demodex count. (23)

In our study, we intend to use Technique B along with the Modified Coston method for counting Demodex mites. These distinct parameters and methodologies contribute to the understanding of demodex blepharitis treatment, but a comprehensive investigation of LLLT in this context is notably absent in the existing literature.

4.4) Eyelid scrubb compliance

Hyun Koo et al. (2012) demonstrated that good compliance with eyelid scrubbing using tea tree oil (TTO) (defined as more than 10 times per week with over 70% compliance) resulted in significant improvements in both Demodex count and the Ocular Surface Disease Index (OSDI) scores. In contrast, the moderate and poor compliance groups did not show statistically significant improvements compared to the control group.(6)

For our study,we will exclude patients with moderate and poor compliance. By focusing on patients who strictly adhere to the treatment protocol, we aim to more accurately assess the effectiveness of the intervention.

4.5) FDA approved medication for Demodex blepharitis : Lotilaner ophthalmic solution 0.25% is the only medication for Demodex blepharitis that was FDA-approved in July 2023(5). A recent meta-analysis showed that lotilaner significantly reduces Demodex mite density and mean collarette scores compared to a placebo(24). However, the cost per bottle is $2,031 for 10 milliliters, and it is not available in Thailand. Consequently, we did not select this medication as an intervention in our study.

4.6) Several imaging modalities are used to diagnose MGD, with meibography emerging as a key tool for evaluating the structure and function of the meibomian glands. Traditional meibography employs infrared light to visualize gland architecture and assess gland dropout, but its reliance on manual interpretation can introduce subjectivity. The advent of artificial intelligence

(AI) in ophthalmology has opened new opportunities for more precise, automated, and objective assessments of meibomian gland health. AI-assisted meibography systems, particularly those based on deep learning algorithms, offer the potential to enhance the accuracy of MGD diagnosis and monitoring by providing automated segmentation, analysis, and grading of meibomian glands(25)

Recent studies have demonstrated the effectiveness of AI in analyzing meibography images to detect structural changes in the glands, including gland dropout and atrophy, with high sensitivity and specificity. Dr. Yuan et al. described the use of deep learning models in meibography for the automatic segmentation of meibomian glands, offering a significant improvement over manual grading in terms of both speed and consistency. Their software integrates multiple steps, including image normalization, automatic segmentation, and manual refinement, to ensure high-quality analysis (18) By providing quantitative data on gland morphology, AI-based tools can assist clinicians in more accurately diagnosing MGD and tailoring treatment plans (26)

Low-level light therapy (LLLT) and intense pulsed light (IPL) have gained traction as adjunctive treatments for MGD. Both therapies aim to improve meibomian gland function by reducing inflammation, improving glandular secretion, and decreasing Demodex mite counts Previous Studies suggest that combining LLLT and IPL with conventional therapies for MGD and blepharitis can lead to better clinical outcomes than monotherapy. However, limited research has evaluated the specific impact of these therapies on meibomian gland structure, especially when assessed using AI-assisted meibography

Our study aims to fill this gap by comparing pre- and post-treatment meibomian gland parameters in patients with Demodex blepharitis, using AI-assisted meibography to evaluate the effectiveness of LLLT, IPL, and combined treatments. By leveraging AI technology, this research intends to offer more objective and quantifiable insights into the impact of these treatments on meibomian gland health, which could ultimately improve patient outcomes in MGD management.

4.7) AI-Assisted Meibography Analysis: Our study plans to utilize an AI-assisted meibography developed system (18) to evaluate and compare the severity of meibomian gland dysfunction before and after low-level light therapy (LLLT) treatment for Demodex blepharitis. What sets this study apart is the integration of advanced AI-driven image analysis, specifically designed to assess the effectiveness of light-based therapies, which are playing an increasingly significant role in the treatment of meibomian gland dysfunction. This novel approach allows for precise, quantitative evaluation of treatment outcomes, offering a cutting-edge method to measure improvements in gland function.

5. Objectives

Study objectives to fulfill the knowledge gap

Primary objective:

To evaluate and compare the efficacy of mechanical with pharmacological treatment, LLLT, IPL and IPL + LLLT with Demodex Mite Count in MGD-related Demodex Blepharitis

Secondary objectives:

To evaluate and compare efficacy of mechanical with pharmacological treatment LLLT, IPL, IPL + LLLT for ocular surface parameters in MGD-related Demodex Blepharitis

6. Hypothesis

Null Hypothesis (H0):

There is no significant difference in the reduction of Demodex mite count among patients with Demodex Blepharitis treated with Low Level Light Therapy (LLLT), Intense Pulse Light Therapy (IPL), Combined LLLT + IPL, and Mechanical with pharmacological Treatment.

Alternative Hypothesis (H1):

There is a significant difference in the reduction of Demodex mite count among patients with Demodex Blepharitis treated with at least one of the following therapies: Low Level Light Therapy (LLLT), Intense Pulse Light Therapy (IPL), Combined LLLT + IPL, greater than Mechanical with pharmacological Treatment.

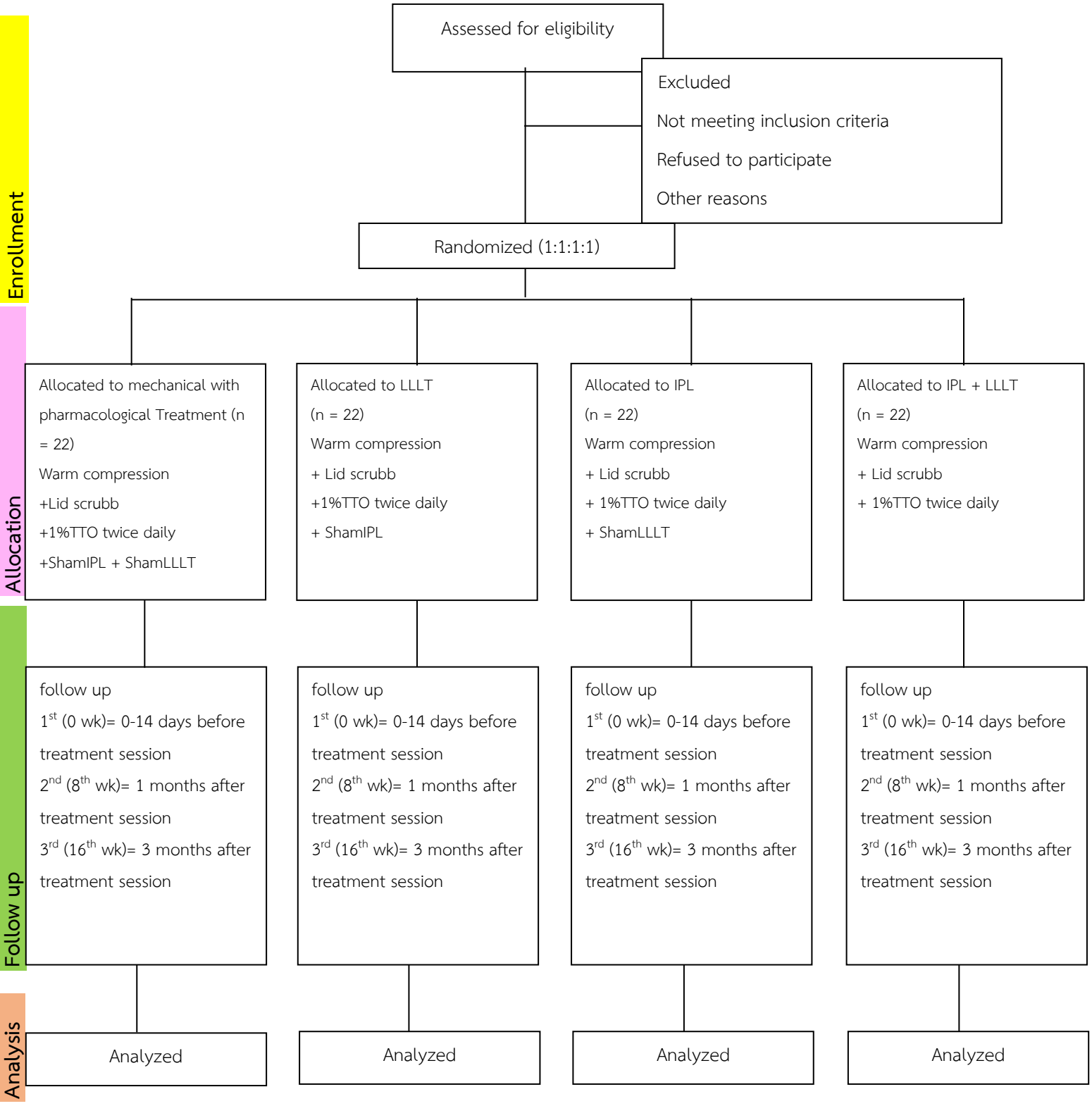
Hypotheses

- LLLT or IPL, will be equally effective in reducing Demodex mite count and improving ocular surface parameters.
- IPL + LLLT will be more effective than LLLT or IPL alone in reducing Demodex mite count and improving ocular surface parameters.

7. Keywords

Demodex blepharitis, MGD, LLLT, IPL, Demodex mite count, Demodex mite eradication, Ocular surface parameters

Conceptual framework



Variables

Independent variables:

the different treatment modalities:

1.Low Level Light Therapy (LLLT)

2.Intense Pulse Light Therapy (IPL)

3.Combined LLLT + IPL

4.Mechanical with pharmacological treatment

Dependent variables: Mean Demodex Count, MGD Staging, OSDI Questionnaire, Meibum Quality, Meibum Expressibility, Telangiectasia Grading (0-3), Surface Staining (NEI Grading System), % Meibomian Gland Loss (MGL), Lipid Layer Thickness, Noninvasive Break-Up Time (NIBUT), Tear Meniscus Height (TMH),

8. Research Design

Single Center, 2x2 Factorial Randomized Control Trials

9. Research Methodology

Target Population: Demodex blepharitis patients at the Department of Ophthalmology, King Chulalongkorn Memorial Hospital.

This is a single-center, prospective, 2x2 factorial Randomized Control Trials involving outpatients with Demodex blepharitis presenting at the Department of Ophthalmology, King Chulalongkorn Memorial Hospital (KCMH) from Nov 2024 to Nov 2025.

The approach to participants: involves patients under the care of the research physician. They are recruited through advertisements and contacted through the patient's physician to introduce the researcher and seek volunteer participation.

The inclusion criteria consist of:

1. All patients who received a definite diagnosis of demodex blepharitis based on cylindrical dandruff at the base of the lashes and the presence of at least one alive demodex mites on eyelid margin by microscopy
2. Age >18 years.
3. Patients who can understand and follow the study instructions and can adhere to the scheduled follow-up plan.

The exclusion criteria consist of:

1. Patients who received previous treatment IPL or LLLT
2. Poor compliance with eyelid scrubbing using tea tree oil (TTO)
3. Dead demodex mite on lash.
4. Contraindication of IPL: Skin Fitzpatrick scale V/VI, patients with pigmented lesions on the treatment area (Eyelid, Cheek).
5. Pregnancy or breastfeeding.
6. Active Skin disease: facial skin cancer, graft-versus-host disease, systemic lupus erythaematosus
7. Active eye infection: recurrent herpes simplex
8. Other lid disease ocular trauma, ocular deformity scar, exophthalmos, eyelid insufficiency
9. Patients with pigmented lesion on area of treatment.

We will use computer-generated random number allocation into 4 groups in a 1:1:1:1 ratio including seed number, with the outcome accessor blind and participant blind with allocation concealment:

1.) ShamLLLT + ShamIPL
2.) LLLT + ShamIPL
3.) IPL + ShamLLLT
4.) LLLT + IPL

***All groups receive mechanical with pharmacological treatment (Warm compression + Lid hygiene + 1% TTO) for 90 days with google form logbook for record compliance and prevent co-interventional treatments.

***All group with participant/investigator/interpreter blind.

Blinding and Allocation Concealment in Research Team:

1st Research Assistant : Allocates patients according to computer-generated random number allocation, ensuring allocation concealment, Evaluated VA , IOP , OSDI score by reads the questionnaire to the participant

2nd Research Assistant (The treatment administrator) : Administers IPL, LLLT, or sham treatments.

3rd Research Assistant : Conducts assessments using Keratograph and Lipiview2.

Main Researcher : Evaluates primary and secondary outcomes using case record forms, remaining blind to the treatment groups.

Intervention Description

Mechanical with pharmacological treatment : Warm compression 15 minutes + Lid hygiene + 1% TTO for 90 days

1%TTO : OCuSOFT Oust Demodex Eyelid Cleanser Foam Bottle 50ml (OCuSOFT, Richmond, TX) is the only commercially available medication in Thailand that contains 1% tea tree oil.

IPL : The Eye-light device (Espansione Marketing S.p.A., Bologna, Italy,waveleggths 1,200-1,500 nm, Delivery system 12cm^2) (Thai FDA No 66-2-2-2-0012685) :

- Protective eye shields
- 5 flashes of light were applied for each eye (3 along the inferior orbital rim, 1 at the lateral canthus, and 1 applied horizontally along the inferior orbital rim)
- Therapeutic energy level (10–16 joules/ cm2) based on the degree of skin pigmentation.

LLLT : The blue MY MASK-E (Espansione Marketing S.p.A., Bologna, Italy) (Thai FDA No 66-2-2-2-0012685)

- No eye shields
- Applying a special mask for 15 minutes, once a week for 4 weeks

ShamLLLT : the MY MASK-E (Espansione Marketing S.p.A., Bologna, Italy) – No eye shields, applying a power off special mask for 15 minutes , once a week for 4 weeks

ShamIPL : the Eye-light device (Espansione Marketing S.p.A., Bologna, Italy) - Protective eye shields, 5 time for each eye with flashes of light off.

Epilation Strategies

the Modified Coston method as outlined by Gao et al. in 2005. In this method, two lashes containing cylindrical dandruff (CD) then additional **rotated around itself**
were extracted from each eyelid of every participant using fine forceps. These lashes were then individually placed at opposite ends of a glass slide for observation under a slit-lamp biomicroscope (SL220; Carl Zeiss, Oberkochen, Germany) at a magnification of ×25. This process resulted in four lashes of right eye being prepared across two slides for each participant. A coverslip was applied to each lash, followed by the slow pipetting of 20 μL of saline at the coverslip's edge until it surrounded the lash. Subsequently, 20 μL of 100% alcohol was pipetted at the same edge, extending the counting period for up to 20 minutes to facilitate the migration of embedded Demodex from the CD. The Demodex count was performed thrice under the microscope, and all samples were photographed using standard procedures by the same specialist. The presence of Demodex in at least one of the eight eyelashes was considered Demodex-positive

10. Data Collection:

Primary Outcome:

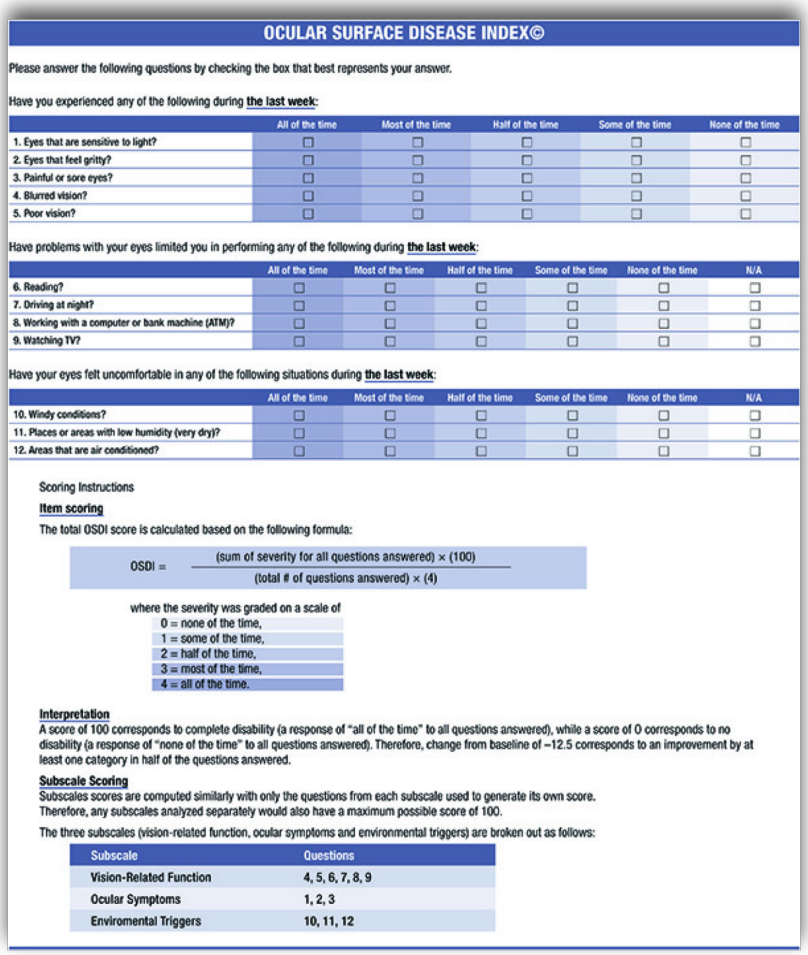
Mean of Demodex Count - the number of Demodex mites per eyelash on a total of 4 collarettes lashes epilation (2 from upper eyelid ,2 from lower eyelid and identification by anatomical imaginary line of nasal and temporal limbus) per right eye in 30 days after end of treatment. (8^{th} week)

Secondary Outcomes:

1.Mean of Demodex Count - per right eye in 90 days after end of treatment.(16^{th} week)

2.MGD staging (Severity)

OSDI questionnaire used to evaluate ocular dry eye symptoms.



OCULAR SURFACE DISEASE INDEX©

Please answer the following questions by checking the box that best represents your answer.

Have you experienced any of the following during **the last week**:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	☐	☐	☐	☐	☐
2. Eyes that feel gritty?	☐	☐	☐	☐	☐
3. Painful or sore eyes?	☐	☐	☐	☐	☐
4. Blurred vision?	☐	☐	☐	☐	☐
5. Poor vision?	☐	☐	☐	☐	☐

Have problems with your eyes limited you in performing any of the following during **the last week**:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	☐	☐	☐	☐	☐	☐
7. Driving at night?	☐	☐	☐	☐	☐	☐
8. Working with a computer or bank machine (ATM)?	☐	☐	☐	☐	☐	☐
9. Watching TV?	☐	☐	☐	☐	☐	☐

Have your eyes felt uncomfortable in any of the following situations during **the last week**:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	☐	☐	☐	☐	☐	☐
11. Places or areas with low humidity (very dry)?	☐	☐	☐	☐	☐	☐
12. Areas that are air conditioned?	☐	☐	☐	☐	☐	☐

Scoring Instructions

Item scoring

The total OSDI score is calculated based on the following formula:

$$\text{OSDI} = \frac{(\text{sum of severity for all questions answered}) \times (100)}{(\text{total \# of questions answered}) \times (4)}$$

where the severity was graded on a scale of

0 = none of the time,
1 = some of the time,
2 = half of the time,
3 = most of the time,
4 = all of the time.

Interpretation

A score of 100 corresponds to complete disability (a response of "all of the time" to all questions answered), while a score of 0 corresponds to no disability (a response of "none of the time" to all questions answered). Therefore, change from baseline of −12.5 corresponds to an improvement by at least one category in half of the questions answered.

Subscale Scoring

Subscales scores are computed similarly with only the questions from each subscale used to generate its own score. Therefore, any subscales analyzed separately would also have a maximum possible score of 100.

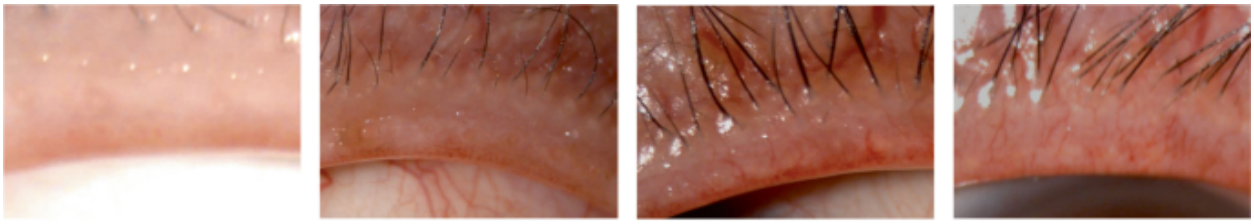
The three subscales (vision-related function, ocular symptoms and environmental triggers) are broken out as follows:

Subscale	Questions
Vision-Related Function	4, 5, 6, 7, 8, 9
Ocular Symptoms	1, 2, 3
Enviromental Triggers	10, 11, 12

Meibum Quality.

Meibum Expressibility.

Telangiectasia grading: 0-3



Grade 0
No findings

Grade 1
Mild telangiectasia

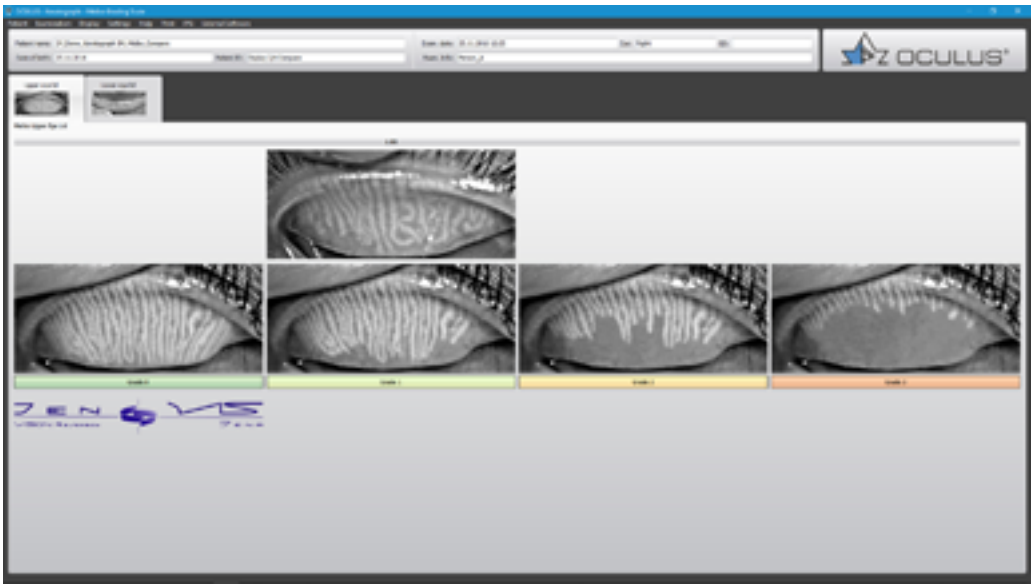
Grade 2
Moderate telangiectasia or redness

Grade 3
Severe telangiectasia or redness

3.Surface Staining: NEI Grading System.

4.Lipid Layer Thickness by LIPIVIEW

5.Noninvasive break-up time (NIBUT), tear meniscus height (TMH) , % Meibomian Gland Loss (MGL) by Keratograph 5M (OCULUS, Germany)



% Meibomian Gland Loss (MGL)

6.Corrected one times baseline , including BCVA, IOP.

7. After complete datacorrection : Meibography images will be masked and analyzed using an AI-assisted program to assess the following:

- Meibomian gland dysfunction staging (severity): MGD severity level and scored with 1-5 according to the 2011 MGD workshop (27) (Figure1)
- Meibomian gland area ratio: The proportion of the total visible meibomian gland area to the overall tarsal area in the eyelid
- Meibomian gland length: The linear measurement from the proximal to distal end of a meibomian gland, reflecting its elongation or shortening due to disease.
- Meibomian gland width: The horizontal width of individual meibomian glands, which helps assess gland atrophy or hypertrophy.

- Meibomian gland diameter deformation index: A metric for measuring irregularities in the shape and thickness of the glands, indicating glandular structural deformation.
- Meibomian gland tortuosity index: A measure of the curvature or twisting of the meibomian glands, used to assess changes in gland architecture.

Meibomian gland signal index: The intensity or brightness of the meibomian gland signal in meibography images, indicating gland density and activity

Visiting schedule:

1st visit - Before treatment within 0-14 days (1st Data correction+Epilation)

2nd-5th visit - 1st – 4th week : Treatment session 1 per week x 4 weeks

- 8th week : 1 month after treatment (2nd Data correction+Epilation)

6th visit - 16th week : 3 months after treatment(3rd Data correction+Epilation)

All data will be collected using Microsoft Excel version 16.65 (Microsoft Inc, USA) and analyzed using SPSS Statistics.

***Informed consent process*:** The study includes an informed consent (Board consent) process that allows the use of research data for future studies without the need for additional consent. . Data will be collected after this process and recorded by the investigator in a manner that subjects cannot be identified

Log book:

Provide a paper logbook to all participants to record compliance and prevent any potential co-intervention by patients or caregivers.

11. Data Analysis and Statistics

Sampling technique and Sample size: N=22 in each arm by

1-Way ANOVA Pairwise, 2-Sided Equality

$$n = 2(\sigma \frac{z_{1-\frac{\alpha}{2\tau}} + z_{1-\beta}}{\mu_A - \mu_B})^2$$

$$1-\beta=\Phi(z-z_{1-\alpha/(2\tau)})+\Phi(-z-z_{1-\alpha/(2\tau)}),\ z=\frac{\mu_A-\mu_B}{\sigma\sqrt{\frac{2}{n}}}$$

$$n=2(3.56\frac{z_{1-\frac{0.05}{2\tau}}+z_{1-\beta}}{3.18-1.59})^2$$

$$1-\beta=\Phi(z-z_{1-\alpha/(2\tau)})+\Phi(-z-z_{1-\alpha/(2\tau)}),\ z=\frac{3.18-1.59}{3.56\sqrt{\frac{2}{n}}}$$

n = Sample size (per pair) = 36, Each arm = 18 , 20% drop out = 21.6 = 22 per arm

Φ = the standard Normal distribution function

Φ −1 = the standard Normal quantile function

a = Type I error, Significance level = 5%

β **=** Type II error, Power (1 – ***β***): 80%

τ = the number of comparisons to be made = 6

Relative difference (*δ*) = 50% (or 0.50) by our expert opinion of minimal clinical significant difference

<u>From previous study(28)</u>

Standard deviation (σ)= Assume an estimated value based on available data or literature (28) = 3.56

(SD Demodex count at baseline)

u_A = 3.18

u_B = 1.59

Statistical analysis, plan, and data management

Demographic and baseline clinical characteristics will be presented as percentages for categorical data and as mean with standard deviation or median with interquartile range (IQR) for continuous data.

For outcome ,Continuous data : Meibomian gland area ratio, Meibomian gland length and width,Meibomian gland diameter deformation index ,Meibomian gland tortuosity index, Meibomian gland signal index should be analyzed using ANOVA, while binary outcomes and ordinal data Meibomian gland dysfunction staging (severity) should be assessed using the chi-square test.

Any unavailable data will be treated as missing and will not be included in any calculations. The report will specify the percentage of missing data in each category. Statistical significance will be determined by a p-value < 0.05.

12.Ethical Consideration

The submission of the research proposal to the Institutional Review Board on Human Research at the Faculty of Medicine, Chulalongkorn University marks a pivotal step in ensuring the ethical oversight and approval of the prospective Randomized Control Trial (RCT)

Respect for Person:

Informed Consent: The principle of respect for persons underscores the importance of obtaining informed consent. Ensuring that participants have a clear understanding of the study's purpose, procedures, potential risks, and benefits is crucial.

Researchers employ transparent and comprehensive communication in the informed consent process, elucidating the randomization process, details of each intervention, and the freedom to withdraw at any point without repercussions.

Researchers will provide comprehensive information to ensure that volunteers understand the research well and make independent decisions regarding participation, without coercion or undue influence. If volunteers have legal representatives or proxies, they will be the ones reviewing the informed consent document and providing witness signatures. Researchers will seek permission from the hospital director at Chulalongkorn Hospital and will anonymize data using codes without disclosing patients' names, surnames, or hospital identification numbers.

Beneficence/Non-maleficence:

Risk-Benefit Assessment: Ethical research necessitates a careful evaluation of potential benefits against risks. Researchers must strive to maximize the positive outcomes of the study while minimizing any harm to participants.

Continuous monitoring of the interventions for unexpected risks and a commitment to modify or halt the trial if harms outweigh benefits are imperative to uphold the principles of beneficence and non-maleficence.

Patients will benefit from receiving treatment for Demodex Blepharitis through eyelid scrubbing + 1% TTO (tea tree oil) and eyelid scrubbing + Low-Level Blue Light Therapy (LLLT) + Intense Pulsed Light (IPL) performed by specialized ophthalmologists. Volunteers will not receive any other benefits from participating in the research. All trial procedures will be tested by researchers and the team beforehand to identify potential issues. Safety will be paramount during the experiment, with basic first aid procedures in place and guidelines for referring participants in case of injuries or adverse effects

Justice:

Equitable Participant Selection: Justice demands fairness in participant selection, avoiding bias or discrimination. All eligible individuals should have an equal opportunity to participate, promoting inclusivity.

Researchers design the recruitment process to ensure diversity and representation across various demographics. The selection criteria based on scientific relevance, fostering inclusivity in the study population.

This research has clear inclusion and exclusion criteria, equally distributes risks and benefits, and employs a stratified study group allocation method.

Equitable Access to Benefits: The principle of justice extends to ensuring that the benefits resulting from the study are accessible to a broad population, preventing disparities in access to potential interventions.

Researchers proactively consider the implications of the study outcomes and work towards facilitating equal access to any effective interventions emerging from the trial, minimizing healthcare disparities.

Outcomes

Primary Outcome: Mean of Demodex Count - the number of Demodex mites per eyelash on a total of 4 collarettes lashes epilation per one eye in 30 days after end of treatment.

Secondary Outcomes:

- Mean of Demodex Count - the number of Demodex mites per eyelash on a total of 4 collarettes lashes epilation per one eye in 90 days after end of treatment.
- MGD Stage 1-5.

OSDI questionnaire used to evaluate ocular dry eye symptoms.

Meibum Quality.

Meibum Expressibility.

Telangiectasia grading: 0-3

- Surface Staining: NEI Grading System.
- LIPIVIEW used to measure Lipid Layer Thickness.
- The Keratograph 5M (OCULUS, Germany) used to measure noninvasive break-up time (NIBUT), tear meniscus height (TMH), % Meibomian Gland Loss (MGL)

13. Expected or Anticipated Benefit Gain

This research marks a pioneering and robust investigation into demodex blepharitis globally, utilizing a meticulously designed study approach tailored to the Thai population.

Aims to unveil unprecedented insights into the new less invasive , less session , better compliance optimal treatment modalities for demodex blepharitis. The expected benefits, applications, and utilization of the study findings are multifaceted, offering valuable contributions to both the scientific community and clinical practice.

Contributes to the optimization of light base treatment strategies for demodex blepharitis, helping clinicians tailor interventions based on their relative efficacy.

Provides a foundation for further research on the sustained effects of these interventions and informs the development of extended treatment protocols.

Generates new knowledge that extends beyond demodex mite eradication, encompassing the broader landscape of ocular surface health, ethical considerations in clinical trials, and the application of innovative therapeutic modalities. This knowledge contributes to the ongoing evolution of demodex blepharitis and meibomian gland dysfunction management and sets the stage for further advancements in ocular surface research.

14.Challenges and Limitations

1. Limited Generalizability
    - Population Specificity: The study's focus on the Thai population may limit the generalizability of findings to broader demographic groups, making it challenging to apply results to populations with different genetic, environmental, and cultural backgrounds.
2. Long-Term Effects and Follow-Up
    - Sustainability and Longevity: The relatively short follow-up period (90 days) may restrict the ability to capture long-term effects of the interventions. Extended longitudinal studies are necessary to assess treatment sustainability over time.
3. Blinding Constraints
    - Blinding in Light-Based Interventions: Achieving complete blinding in light-based interventions (IPL and LLLT) is challenging, potentially introducing bias. The visible nature of these treatments makes it difficult to conceal allocation from both participants and investigators.
4. Outcome Measure Subjectivity
    - Subjective Assessments: Reliance on patient-reported symptoms may introduce variability in study outcomes. While objective measures, such as Demodex mite count, offer a more standardized evaluation, they remain subject to interpretation.
5. Adherence and Compliance
    - Patient Adherence: The effectiveness of interventions depends on patient adherence to treatment protocols. Challenges may arise if patients perceive the procedures as burdensome or experience difficulties maintaining compliance.
6. Limitations of AI Technology

- Algorithm Performance: AI-driven meibomian gland analysis is dependent on the quality of algorithms and training data. Variability in clinical conditions and diverse populations may impact the accuracy and effectiveness of AI models.

7. Integrating AI Insights into Clinical Practice

- Clinical Implementation: Translating AI-supported research findings into practical clinical guidelines and integrating them into routine practice requires extensive validation. Additional verification and refinement of study results may be necessary before AI-driven approaches can be widely adopted in clinical settings.

15. Risk and Investigator's Responsibility

1. Patient Privacy Protection:

Risk: The collection of sensitive health information necessitates the protection of patient privacy.
Investigator's Responsibility: The investigator must adhere to data protection regulations, implement robust anonymization practices, and establish secure data storage and transmission procedures to safeguard patient privacy.

2. Participant Discomfort during Interventions:

Risk: Participants undergoing Photobiomodulation (LLLT) and Intense Pulsed Light (IPL) therapies may experience mild discomfort or temporary side effects.
Investigator's Responsibility: The investigator must thoroughly explain potential discomfort during the informed consent process and promptly address any participant concerns or adverse reactions.

3. Selective Reporting Bias:

Risk: There is a risk of selective reporting bias.
Investigator's Responsibility: The investigator must commit to transparently reporting all outcomes, fostering an unbiased and comprehensive understanding of the interventions' efficacy.

4. Bias and Accuracy in AI Analysis

Risk**:** AI models may have inherent biases or limitations that affect the accuracy of meibomian gland analysis, potentially leading to incorrect diagnoses or assessments.

Investigator's Responsibility: Researchers must rigorously monitor and evaluate the performance of AI systems, actively identifying and addressing biases or errors to ensure the reliability and credibility of study outcomes.

16. Timeline

The research will take a total of 12 months to complete.The recruitment of volunteers to participate in the research will begin after the research protocol has been approved by the Research Ethics Committee and the Medical Faculty.

17. Venue of the study

Department of Ophthalmology, King Chulalongkorn Memorial Hospital

18. Tabulation of Research Activities and Timeline

Month	1	2	3-4	5-6	7-8	9-10	11-12
Research design							
Proposal writing & defense							
IRB submission							
Data collection							
Data analysis							
Manuscript writing							
Publication							

New Knowledges

- Contributes to the optimization of light base treatment strategies for demodex blepharitis, helping clinicians tailor interventions based on their relative efficacy.

- Provides a foundation for further research on the sustained effects of these interventions and informs the development of extended treatment protocols.
- Generates new knowledge that extends beyond demodex mite eradication, encompassing the broader landscape of ocular surface health, ethical considerations in clinical trials, and the application of innovative therapeutic modalities. This knowledge contributes to the ongoing evolution of demodex blepharitis and meibomian gland dysfunction management and sets the stage for further advancements in ocular surface research.

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Staging the Severity of MGD and Individual Clinical Parameters

	Severity Level					
	Level 0 Normal	**Level 1 Subclinical**	**Level 2 Symptomatic Minimal**	**Level 3 Symptomatic Mild**	**Level 4 Symptomatic Moderate**	**Level 5 Symptomatic Severe**
Symptom frequency and severity	No symptoms	Asymptomatic or occasional symptoms	Some of the time; precipitated by environmental factors	Half of the time; some limitation of activity	Most of the time; frequent limitation of activity	All of the time Severe/disabling/constant
OSDI grade range (0–100)	0	0–12	0–12	13–22	23–32	33–100
MGD Grade	Clear	Subclinical, nonobvious MGD; altered quality, only on expression; no gland loss	Minimally altered quality of expressed meibum from scattered glands; None to minor gland loss	Mildly altered meibum quality; occasional lid margin signs; mild gland loss	Moderately increased opacity and viscosity of meibum; plugging; increased marginal vascularity; loss of orifice definition; moderate gland loss	Marked, diffuse MGD; cicatricial or noncicatricial; multiple lid margin signs; lid deformity and marked lid margin hyperaemia; Severe gland loss
Quality of expressed meibum grade range 0–3, LL, 8 glands, Range (0–24)[*]	0	1–5	6–10	11–15	16–20	21–24
Treatment of MGD based on symptoms and gland status		+ General advice about MGD, the potential influence of diet, home and work environment	+ Hygienic measures, heat and massage	±Topical ATs	+Oral tetracycline derivatives	± Anti-inflammatories
		±Hygienic measures		± Emollient lubricant or liposomal spray ± Topical azithromycin ± Consider oral tetracycline derivatives		

This table should be read in conjunction with Table 11, which provides a staging scheme for MGD-related *ocular surface disease*. Severity levels for each parameter are graded 1–5. A subclinical severity level has been introduced to accommodate asymptomatic MGD with normal lid margin features (nonobvious MGD) diagnosed only after gland expression. Note that this MGD scoring system does not provide a score for totally obstructed glands. Alternative systems for grading MGD exist and should be considered (see Appendices 5–7). Arita et al.[3] graded meibomian dropout in the combined upper and lower lids, using noninvasive meibography, with a scale range of 0–6 (Table 3). de Paiva et al.[106] used a composite system combining dropout, lid signs and meibum expressibility, with a scale range of 0–11 (Table 3). Korb and Blackie[22] record the number of glands in a zone of 8, which yield a liquid secretion after standardized expression (MGYLS score 0–8). General treatment concepts, summarized here, are adapted from the Report on Management and Therapy. Recommended treatments are additive. At each clinical assessment, lack of response to treatment at the previous level moves treatment to the next level. ±, the decision to use this treatment is based on clinical judgment; +, treatment is recommended at this level. LL, lower lid; OSDI, Ocular Surface Disease Index.

[*]The increase in severity of MGD with increase in grade is denoted by a reduced quality of expressed meibum. Meibum quality (clarity and consistency) is assessed in eight glands of the central third of the lower lid on a 0–3 scale for each gland: 0 = clear; 1 = cloudy; 2 = cloudy with debris; 3 = thick, like toothpaste (total score range 0–24).[22]

Figure1