I-LID 'N LASH® PLUS: A Safe and Effective Product in Managing Demodicosis *in vivo*

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Abstract

The incidence of demodicosis tends to rise with age, varying from 13% in individuals aged 3 to 15 years to 100% in those above 70 years old. Demodicosis plays a significant role in Meibomian Gland dysfunction, making it a crucial therapeutic target. Tea Tree Oil (TTO), derived from the Australian native plant Melaleuca alternifolia, remains the standard of care for demodicosis treatment.

A recent study unveiled that Terpinen-4-ol, a prominent component in TTO, demonstrates lethality for human immortalized Meibomian Gland cell lines in vitro. The current study aims to evaluate the in vivo safety and effectiveness of I-LID 'N LASH® PLUS, which contains 5% TTO. Twenty-one dry eye patients experiencing demodicosis used I-LID 'N LASH® PLUS twice daily for 30 to 45 days and were evaluated for signs and symptoms, as well as meibography to determine the safety and the efficacy of this product in vivo.

Results indicate that I-LID 'N LASH® PLUS significantly decreased the Average Collarettes Score (or Average Cylindrical dandruff Score) and lowered the Ocular Surface Disease Index (OSDI) score. This was accompanied by a non-significant increase in Tear Break Up Time (TBUT), confirming the efficacy of the product. The results also confirm the safety of the product, as no Meibomian gland loss was observed, and no adverse events were reported during the study's duration.

Introduction:

Demodicosis is a skin condition characterized by an overpopulation of Demodex mites.[1] These microscopic mites, known as Demodex folliculorum and Demodex brevis typically inhabit hair follicles and sebaceous glands, respectively.[2] They are naturally present on human skin and hair, including eyelids and eyelashes. Demodex mites are generally harmless and are a normal part of the skin's microflora, however, an excessive proliferation of these mites can lead to demodicosis.[1] The prevalence of Demodex mites on the human skin tends to increase with age. The reasons for this age-related increase in prevalence are not entirely clear but may be related to factors such as changes in the sebum composition and increased activity of sebaceous glands which occur with age.[3-4] These species are responsible for a range of eye-related issues, such as blepharitis, rosacea, keratitis, meibomian gland dysfunction, and inflammatory symptoms affecting the eyelids.[5-6] Demodicosis has recently been demonstrated to have a solid link to Dry Eye disease (DED), high Ocular Surface Disease Index score (OSDI), high Schirmer values, shorter TBUT in general but also to Meibomian Gland Dysfunction. [4,7-9] The primary and more achievable objective in treating demodicosis is to decrease the mite population since complete elimination is nearly impossible. Various treatment options are recommended to patients based on the severity of symptoms and the extent of mite infestation.[10]

At present, various treatments exist for demodicosis management, encompassing topical and systemic approaches, including Okra extract, Thai herbal essential oils, ivermectin, metronidazole, and TTO. However, TTO and its derivatives continue to be the globally recognized standard of care, despite the potential for ocular irritation at higher concentrations.[11-13] TTO probably has multiple modes of action as the oil contains multiple molecular species with different potentially active ingredients.[14] It has been demonstrated that terpinen-4-ol is the most abundant and possibly the most powerful molecular species in killing Demodex even at 1% concentration when diluted in mineral oil.[15] The exact mechanism of action for TTO in killing Demodex and improving the Demodex

blepharitis condition is not very well elucidated, nevertheless, it is suggested that TTO induces the migration of Demodex mites out of the skin, potentially facilitating the effectiveness of treatments against them. Additionally, TTO may act on Demodex by competitively inhibiting acetylcholinesterases; exerting antibacterial effects against Demodex thus affecting the Demodex infestation-associated immune reaction and possessing anti-inflammatory properties that inhibit the production of pro-inflammatory proteins (e.g., tumour necrosis factor-alpha, interleukin-8) and oxygen-derived reactive species (superoxide), thereby contributing to the resolution of some of the accompanying conditions. [14,16-17] These effects have also been theorized to be dose-dependent, with a 50% concentration of TTO exerting a direct killing effect on the mites, while a 5% concentration may disrupt their reproductive life cycle by impeding mating.[16]

A recent study claimed that terpinen-4-ol is lethal to Immortalized Human Meibomian Gland Epithelial Cells (IHMGECs) in vitro in a terpinen-4-ol concentration-dependent manner.[18] We understand the importance of this novel research, however, this research has been done using only terpinene-4-ol, excluding all the other components of TTO that may help cell survival. Moreover, this study has been conducted using only one cell line that is genetically modified to be transformed into an immortalized cell line, capable of maintaining its proliferation in vitro. All tests indicated in this study, done in an in vitro setting, may provide some scientific support but does not reflect the actual cellular behaviour in vivo. Finally, this study is lacking an *in vivo* model to confirm this observation.

Our product, I-LID 'N LASH® PLUS, contains 5% TTO and has been a market leader since its first launch almost twenty years ago. The product is offered as a ready-to-use pre-soaked wipe which has been proven to hydrate and soothe the skin due to its high composition of water, hyaluronic acid, and glycerin. This product has an excellent safety profile and has been ophthalmologist and dermatologist tested. In light of the recent publication highlighting that terpinen-4-ol might have toxic effects, we have conducted this study to investigate and determine the safety and efficacy of our product in vivo in a clinical setting. Dry eye patients who concomitantly suffer from Demodicosis have been instructed to use the I-LID 'N LASH® PLUS twice daily for 30-45 days. Patients have been assessed for Demodex, TBUT, symptomatology and meibography before and after the treatment to investigate the effect of the treatment on demodicosis and its effect on Dry eye and to determine if our product is safe in actual in vivo clinical application.

Method:

Study design:

This clinical investigation is a prospective, multicentered, non-randomized, single-group, interventional study conducted at two centers across Canada to assess the effectiveness and safety of I-LID 'N LASH® PLUS. Dry eye patients underwent an evaluation to confirm demodicosis. The verification involved the use of the TearCheck® device to observe the presence of abundant cylindrical dandruff/collarettes. Accordingly, inclusion criteria encompassed dry eye patients with evidence of Demodex around the eye, i.e., a confirmed presence of cylindrical dandruff in patients who are either symptomatic or asymptomatic, and who are willing and consent to participate in the study. Exclusion criteria encompassed individuals under eighteen years old, and those incapable of providing informed consent, and individuals with known allergies to any I-LID 'N LASH® PLUS ingredients or lacking signs of confirmed demodicosis.

Clinical Assessment:

Patients underwent a thorough eye examination utilizing the TearCheck® device to detect cylindrical dandruff/collarettes. These collarettes, debris resulting from mite accumulation at the eyelash roots, served as an indicator of Demodex's presence. Subsequently, the abundance of Demodex was gauged by counting cylindrical dandruff/collarettes per field, called Average Collarettes Scores, and the severity of infestation was assessed using a score chart (Table 1) that outlines the intensity of detected eyelid collarettes done by counting the number cylindrical

dandruff/collarettes of per 10 eyelid lashes per field. This new severity assessment scale is called the Collarettes Severity Scale. The assessment involved scanning both upper and lower eyelids for Demodex, with two fields of TearCheck® images recorded for each margin.

Table 1: A score chart used to evaluate the intensity of collarettes obtained using the Demodex exam on TearCheck®

Item	Collarettes Severity Scale (collarettes per 10 eyelid lashes)			
Eyelid collarettes	0 = None	1-3 Mild	4-6 Moderate	7-10 Severe

Following this, either the Antares device or visual assessment of meibomian glands under slit lamp technic were utilized/ followed to evaluate the percentage area loss of meibomian glands designated as % MG area loss. The recorded % MG area loss serves as a clinical parameter. To ensure accuracy and proper assessment of safety, both upper and lower eyelids were assessed for the % MG area loss.

Additional evaluations involved TBUT to gauge tear film strength. TBUT was assessed either using the Antares device or manually using sodium fluorescein with the slit lamp. Finally, the OSDI – publicly available was also used to evaluate the impact of demodicosis and DED on patients' symptoms.

Treatment and re-assessment:

Patients with a confirmed presence of Demodex were enrolled and instructed to use the I-LID 'N LASH® PLUS, by using 1 wipe per eye, twice a day, for the duration of the study – 42 to 45 days which represents two life cycles of Demodex mites. Patients were also instructed to use a logbook to track their use of the product and ensure compliance. Patients were instructed to continue their regular non-demodicosis treatment of any associated ocular or non-ocular conditions.

At the end of the trial (42 - 45 days), all the above-mentioned pre-treatment assessment tests were repeated on the participants, which included Demodex assessment, % MG area loss, TBUT, and OSDI).

Statistical analysis:

A one-tailed hypothesis testing was employed in this study to evaluate the efficacy (denoted by the intensity of collarettes around the base of the eyelash and TBUT) and safety by % MG area loss, of I-LID 'N LASH® PLUS.

Results:

A cohort of twenty-one individuals, comprising nine males and twelve females were successfully enrolled and finished the study. The age of the participants ranged from thirty-eight to ninety-three years (average 65.7 years old), ensuring representation across various age groups and accounting for potential differences in treatment resistance associated with aging. Uncompliant with the study protocol, patients used the I-LID 'N LASH® PLUS twice daily for at least 30 days and up to 45 days, which reflects the actual compliance rate of patients.

The Average Collarette Score demonstrated a significant (p=0,00009, ***) 42% reduction in the whole cohort assessment (Figure 1.a & 1.c). These results are also confirmed alternatively by demonstrating a reduction in the Collarettes Severity Scale where patients shifted from Moderate and Severe severity grades to None or Mild severity grades (Figure 1.b).

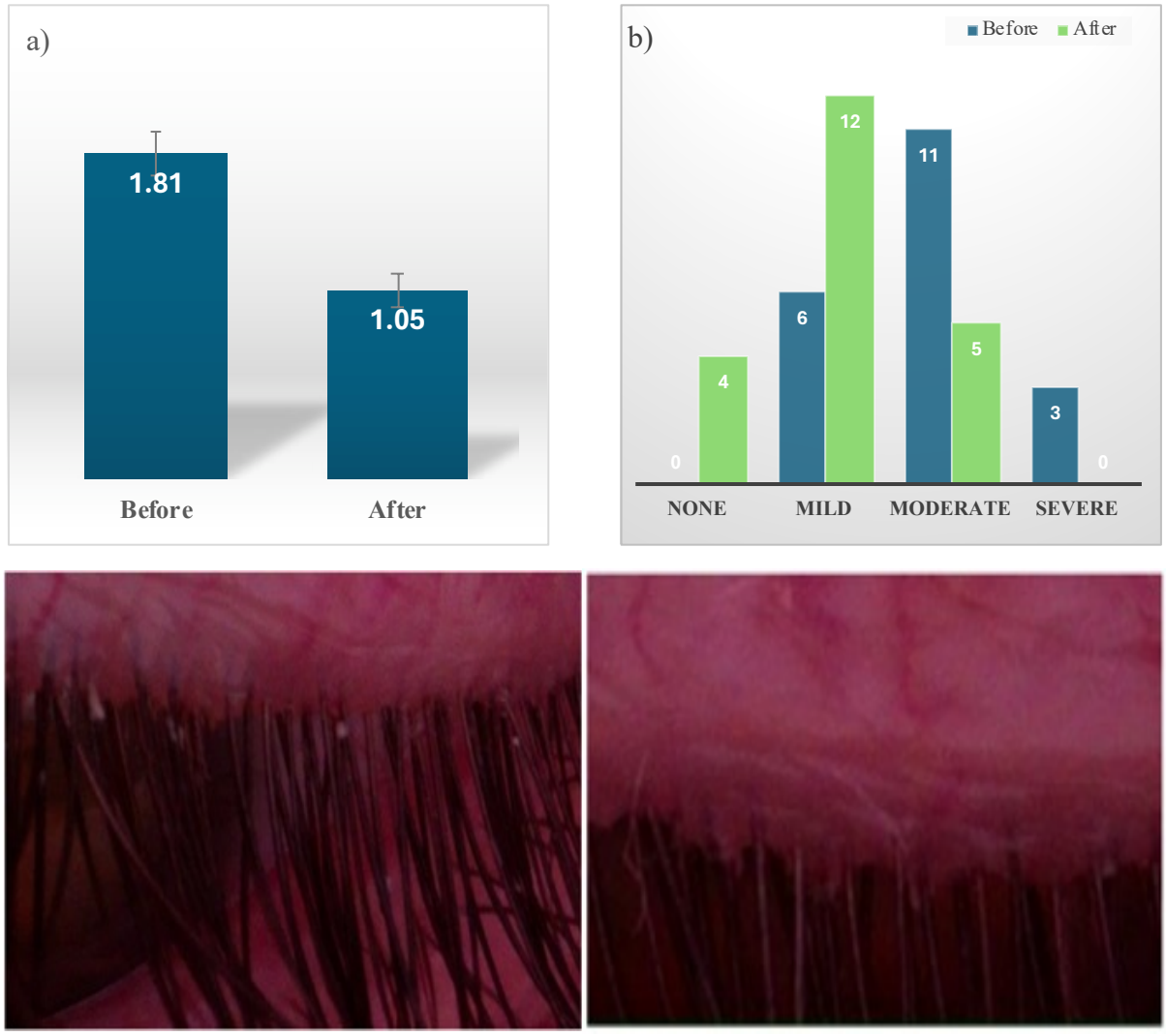

Figure 1: Cylindrical dandruff/collarettes and severity assessment. (a) the average collarette score before and after treatment (p = 0.00009, ***, bars represent the standard error), (b) Collarettes severity scale before and after treatment, and (c) Images obtained with Demodex assessment function of the TearCheck to evaluate cylindrical dandruff/collarettes before and after treatment.

The results also reported a significant (p=0.001, ***) 57% improvement in overall symptomatology, seen by a decrease in OSDI score (Figure 2). Another improvement was noticed in the tear stability seen by a non-significant increase in Tear Break-Up Time (Figure 3).

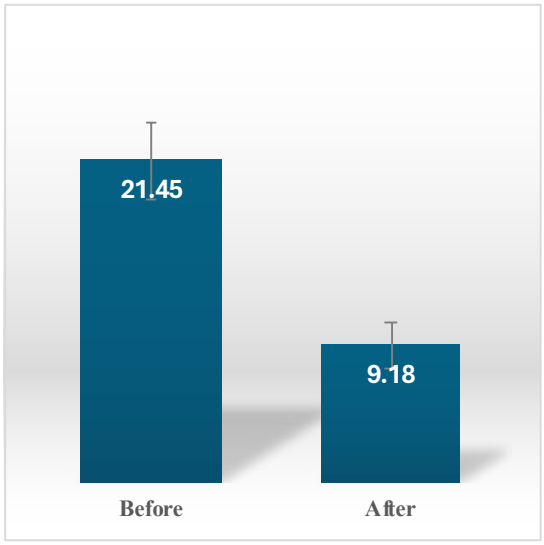

Figure 2: Ocular Surface Disease Index (OSDI) average score before and after the treatment (p = 0.00009, ***, bars represent the standard error).

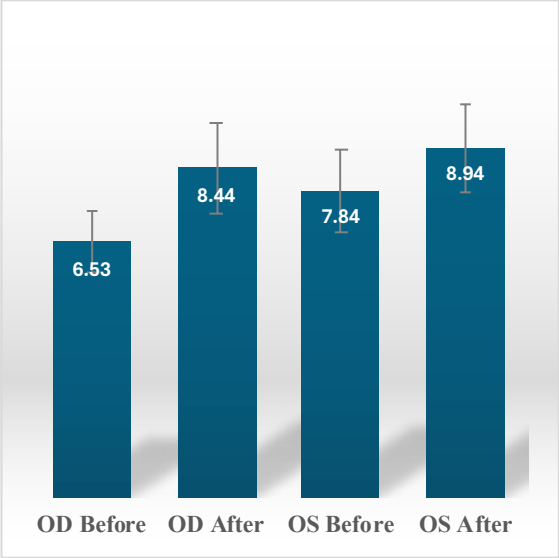

Figure 3: Average Non-invasive Tear Break Time (NITBUT) scores show non-significant increase before and after the treatment for the right eye (OD) and left eye (OS), bars represent the standard error.

None of the subjects reported any treatment-related adverse event aside from the fact that meibography revealed no significant increase in the percentage area loss of meibomian glands (Figure 4).

Figure 4: Meibography (% area lost) test shows no significant increase in the percentage area loss of meibomian glands before and after the treatment, bars represent the standard error.

Subgroup analysis of patients aged 60 years or less revealed a significant (p=0.006, ***) 60% reduction in the Average Collarettes Score (Figure 5.a). These results are also confirmed alternatively by demonstrating the reduction in the Collarettes Severity Scale where patients shifted from Moderate and Severe grades to Mild grade (Figure 5.a) and a significant (p=0.007, ***) 73 % improvement in overall symptomatology, seen by a decrease in OSDI score (Figure 5.c).

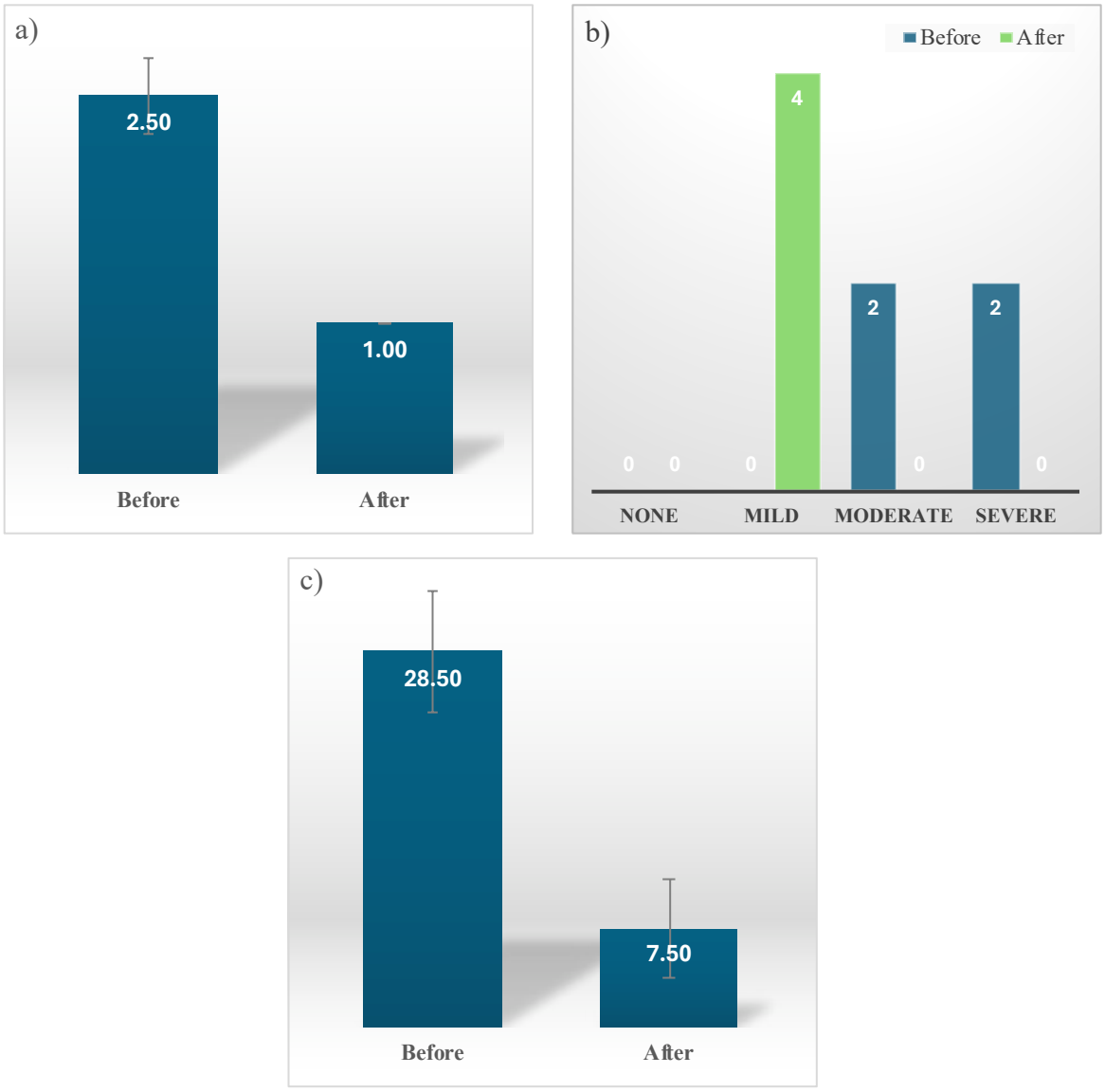

Figure 5: Cylindrical dandruff/collarettes and severity assessment. (a) the average collarette Score before and after the treatment, ($p = 0.006$, ***, bars represent the standard error) (b) the average Collarettes Severity Scale before and after the treatment, and (c) the Ocular Surface Disease Index (OSDI) score before and after the treatment ($p = 0.007$, ***, bars represent the standard error).

Discussion:

This study was conducted to investigate the efficacy of I-LID 'N LASH® PLUS containing 5% TTO in managing Demodicosis by reducing the collarettes/cylindrical dandruff counts, improving tear film stability and proving the safety on the Meibomian Glands, *in vivo*.

Demodicosis and dry eye are two distinct yet interconnected ocular conditions that can coexist and contribute to ocular discomfort. Demodicosis refers to an infestation by Demodex mites, specifically Demodex folliculorum and Demodex brevis, on the eyelashes and in the sebaceous glands of the eyelids. These microscopic mites can lead to various eye-related issues, including inflammation of the eyelids (blepharitis), meibomian gland dysfunction, and the production of cylindrical dandruff or collarettes. The presence of Demodex mites may exacerbate existing eye conditions or contribute to the development of new ones.

I-LID 'N LASH® PLUS has been shown to effectively reduce the count of collarettes/cylindrical dandruff in the entire study group, with a random count on eyelashes under magnification fields, revealing a significant 42% reduction. This underscores the product's capability to decrease the Demodex count. The average collarette count

was diminished from 1.8 to 1 within just 30 to 45 days of treatment with I-LID 'N LASH® PLUS. Notably, the treatment duration observed by patients was shorter than the recommended 42 to 45 days, aligning with two mite life cycles. This deviation reflects real-life patient behaviour as opposed to ideal clinical settings. The persistence of some collarettes/cylindrical dandruff may be attributed to this variance and underscores the importance of clinicians closely monitoring patient compliance to optimize treatment outcomes.

The decrease in collarettes/cylindrical dandruff resulted in a transition of the infestation severity or intensity to a lower grade. Consequently, a higher number of patients were categorized as MILD, and notably, four patients were classified as having NONE on the Collarettes Severity Scale. This outcome confirms the efficacy of the treatment, as assessed through a more accurate counting method—namely, the number of collarettes/cylindrical dandruff per ten eyelashes—which was specifically designed for this study.

The improvement in demodicosis condition was accompanied by a significant 57% improvement in overall symptomatology, as evidenced by a reduction in OSDI score across the entire cohort. This suggests that addressing demodicosis positively influences overall symptoms. This improvement shifted patients from a state of almost moderate symptomatology (OSDI score of 21.4) to being completely asymptomatic (OSDI score of 9.1). With this in mind, we believe that assessing and managing demodicosis is a fundamental step in comprehensive dry eye management.

While we anticipated observing a reflection of this improvement in tear film stability, the TBUT assessment indicated only a numerical enhancement. We believe that increasing the sample size and encouraging patients to adhere strictly to a 42 to 45-day treatment regimen could potentially lead to an increase in TBUT, possibly reaching statistical significance.

Recognizing that demodicosis tends to be more prevalent and resistant to treatment in elderly patients, we conducted a sub-group analysis focusing on individuals 60 years of age and younger to assess the product's efficacy in a younger demographic. The findings revealed a remarkable improvement in demodicosis, with the Average Collarettes Score significantly decreasing by 60%, reducing the average count from 2.5 to 1 collarette per field. Once again, I-LID 'N LASH® PLUS demonstrated robust efficacy, leading to a significant 73% reduction in OSDI score. This shift brought patients from a MODERATE symptomatology level (OSDI score of 28.5) to being completely asymptomatic (OSDI score of 7.5). These outcomes underscore the genuine effectiveness of I-LID 'N LASH® PLUS in managing demodicosis and suggest the potential need for repeated or extended treatment in populations above the age of 60 years.

Wang et al. (2020) suggested that terpinen-4-ol, a major component in TTO, exhibits lethality for human immortalized Meibomian Gland cell lines in vitro. While this study is innovative, it is crucial to note its limitations, as it was conducted in vitro, utilizing only one genetically modified, immortalized cell line. Furthermore, the study exclusively focused on terpinen-4-ol, whereas TTO extract contains numerous other ingredients that may exert varying effects on cell viability and may not be directly extrapolated to the in vivo clinical reality.

Despite TTO being the standard of care for demodicosis management, our product, along with several others featuring higher TTO concentrations, have been in the market for many years, successfully treating millions of patients worldwide. Notably, there has been no reported case of meibomian gland loss following treatment. Nevertheless, we conducted an assessment of our product's safety, and the results indicate no increase in meibomian gland percentage area loss. In fact, a slight decrease was observed. This decrease may be within the error of measurement and may be attributed to technical variability or potentially improved gland structure due to demodicosis management, such as reduced inflammation, leading to an improved outcome in meibography assessment. Noteworthy, the study didn't report any Adverse Events.

Study limitation:

Subsequent investigations may wish to address certain constraints within this study, including the relatively small

sample size and the need to assess the product's performance over prolonged and repeated treatments. Given the potential recurrence of demodicosis, repeated treatment scenarios would provide valuable insights into the sustained efficacy and safety of TTO and this product.

Conclusion:

In summary, our findings indicate that I-LID 'N LASH® PLUS demonstrates high efficacy in the management of demodicosis. This could prove particularly advantageous for individuals experiencing concurrent issues of both dry eye and demodicosis. In addition, this product proved safe for the duration of this treatment by not exhibiting any meaningful reduction in the % MG area loss.

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