Onychomycosis (OM) is a chronic fungal infection of the nail plate, nail bed, or both which affects nearly 10% of the general population under age 60 with the prevalence sharply rising to 30% over age 60. [1,2]. It has been estimated that Medicare in the United States spends approximately $43 million dollars annually to treat OM [1,2]. OM consists of three sub-classifications which are determined by origin and displacement pattern of the infection. Sub-classifications include distal-lateral subungual OM (DLSO), proximal subungual OM (PSO), and superficial white OM (SWO). DLSO has been recognized as the most prevalent form, which presents with a yellow or white discoloration and keratinous debris accumulation that initiates along the distal and lateral undersurface of the nail plate displacing towards the proximal end. SWO has been clinically described by a chalky white material casing the nail surface. The least prevalent is PSO, which manifests in predominately in immunecomprised patients. When having been left untreated, nail deformity can yield deleterious effects on a patient's quality of life as a consequence to the pain that results from nail pressure. In addition to the physical limitations imposed by the disease, psychosocial consequences have been documented in nearly 74% of OM patients citing avoidance of intimacy and embarrassment [2].

Clinical management of OM requires a multifaceted approach coupling therapeutic strategies with appropriate nail hygiene. Adherence to quality foot care such as maintaining dry feet, trimming nails, and utilization of adequate footwear can help support the efficacy of chemical, antifungal, or mechanical treatments. Presently, the application of a topical or oral antifungal represents the mainstay for OM care. Oral medications include the azole family (Fluconazole, Itraconazole, Miconazole, and

Ketoconazole), fluorinated pyrimidine (Flucytosine), griseofluvin, and allylamine Terbinafine. A 2004 meta-analysis of 36 studies has reported terbinafine has the highest cure rate of 76% compared to 60% for griseofluvin, 48% for fluconazole, 59% for itraconazole. The common adverse events that have been reported for oral antifungal therapies include nausea, diarrhea and abdominal pain. Although quite rare, hepatotoxicity has been reported as a potential side-effect, which has required patients undergo liver function tests at baseline and weeks 4 or 6. Furthermore, terbinafine has been shown to modulate the pharmacokinetics of other drugs by inhibiting cytochrome P2D6, an enzyme responsible for drug metabolism.

Development of innovative therapeutic approaches has become an ever-evolving discipline as more potent and selective pharmacophores are required to improve the proficiency of a treatment while minimizing toxicity. One therapeutic approach that has shown promising outcomes for the treatment of OM without a recorded side-effect has been low-level laser therapy (LLLT). Photomedicine has become an accepted modality across numerous medical disciplines and is supported by a wealth of peer-reviewed histological and clinical publications. LLLT, when delivered with specific output parameters, has exhibited anti-microbial effects as well as strengthening the body's natural defense mechanisms by upregulating specific immunological factors. Without having undergone a well-defined clinical investigation, LLLT has not been recognized as an efficacious alternative to mainstay OM therapies. Therefore, the intention of this pilot investigation was to evaluate the clinical utility of a dual-diode low level laser device emitting 405nm and 635nm for the treatment of DLSO.

Methods:

A prospective, non-randomized, non-controlled study was conducted from February 2010 to February 2011. Participants who presented with typical clinical patterns of DLSO received treatment with a low-level laser device. A total of 168 toes were enrolled and treated which included patients presenting with clinical symptoms of OM on multiple toes unilaterally and/or bilaterally. All participants were deemed eligible to participate in the study based on their individual history and the physical criteria set-up by all participating clinics. The female to male ratio was 2:1 with 112 females and 56 males. The average age for all subjects was 59.3 years with average duration of disease of 8.19 years. All participants agreed to share their clinical outcomes for publication and educational purposes.

Prior to the treatment administration phase, all participants were provided with a detailed explanation of the treatment procedure and understood that their outcomes, absent of any protected health information, could only be used for peer-reviewed publication purposes. Participants were excluded from the study based on the following criteria: active illness, severe cardiovascular disease, terminal disease (i.e. cancer), liver or kidney disease, pregnancy, breast-feeding, disease of the thyroid gland, uncontrolled diabetes, disease of the cardiovascular system, drastic weight fluctuations, and other ailments impacting their overall quality of health.

The clinical data obtained for this study were obtained from participants who actively sought the services of a clinician providing treatments for OM, i.e., participants were not openly recruited. Participants were not offered any form of compensation for participation in the investigation. Furthermore, all participants were financially responsible for the procedure or related evaluations along with any travel expenses.

Treating clinicians were not financially responsible for the device as they were provided a device for clinical trial purposes only. Participants were asked to abstain from applying or receiving any other treatment to promote resolution or improvement of OM which included over-the-counter, prescription, or natural remedies. Subjects were provided with a Tineacide antifungal spray to be applied to shoes only as a means to prevent re-infection. Patients were provided with appropriate instructions regarding the use of the spray and were directly informed not to apply to the infected toe(s).

Intervention

Participants received treatment with a dual-diode laser device consisting of two independent diode laser heads emitting a divergent beam (FX405, Erchonia Medical Inc.). One diode emitted a 635nm (red) laser light while the other diode emitted a 405nm laser light. Each diode had an output intensity of 17.5-mW output

The Tineacide antifungal spray (Manufacturer) contained 5% undecylenic acid and was applied once a week corresponding shoe(s).

Study Design

Prior to the treatment administration phase baseline photographs were taken (Olympus E Volt Camera) at variable distance which focused on the toe(s) infected for each extremity. The images were uploaded and the percentage of nail presenting with clinical symptoms of OM was determined by an independent investigator. Post treatment administration phase images were acquired at each new month for a total of 12 months. Patients were granted flexibility regarding post-procedure follow-up visits as a method to minimize study ‘drop-outs.’ Patients were asked to attend as many post-procedure follow-ups and received monthly telephone calls in order to remind subjects

to schedule a follow-up visit. All 168 subjects received at least one post-procedure evaluation between 2 and 13 months.

The treatment phase was initiated following baseline photographs. The treatment phase consisted of a single treatment per week for two weeks for a total of two treatments. Each foot was treated for 12 minutes independently regardless if infection was bilateral. Subjects were asked to remove shoes and socks and were positioned in a standard waiting-room chair with both feet positioned flat on the floor surface. Participants placed a single foot inside the device where their foot was positioned exactly 6 inches below the light-emitting apparatus. The device administered treatment over all five toes concurrently irrespective of infection on adjacent toes. No debridement of the nail was performed as well as no adjunctive agents applied before treatment. After 12 minutes, participants removed their foot the device was cleaned using 5% benzoyl peroxide, and subjects were asked to place their other foot inside the treatment device. Following completion of the treatment patients were educated regarding the appropriate application of Tineacide and provided a new pair of socks.

Subjects were asked to return one week later for the second and final treatment. Subjects were informed to apply Tineacide once-a-week to all shoes.

Data Analysis

No primary or secondary outcomes were established before the start of the trial. Baseline assessments were conducted to evaluate the percentage of nail involved with OM. No defined endpoint evaluation time points were established; therefore, subjects were asked to return between months 2 and 13 following the treatment administration

phase. The percent involvement at the post-procedure evaluation phase was compared with the baseline percent of involvement to determine the percent improvement in nail clarity.

A 2-sample t-test was performed for correlated samples of the change from baseline measurements to study endpoint evaluating the percent of nail involved with onychomycosis. Furthermore, a one-way ANOVA for 4 independent samples was conducted to evaluate the percent improvement at the study endpoint by category of percent nail involved with onychomycosis at baseline. The four categories of percent nail involved with OM included ≤ 50%, 51-79%, 80-95%, and 100% involvement. A one-way ANOVA was also performed for 5 independent samples evaluating the data grouped according to 5 individual post-procedure evaluation month categories. The post-procedure evaluation categories included 2-4, 4.5-5.5, 6, 7-8, and 9-13 months.

Results

Toes presenting with 100% nail involvement represented 45.8% of all toes examined in this study with the remaining 91 toes distributed across three separate categories. The range of percent involvement at baseline ranged between 20% and 100%; therefore, toes were divided into four groups determined by their baseline percent of involvement (Table 1).

Table 1: Categories of percent nail involved with onychomycosis at baseline (n =168)

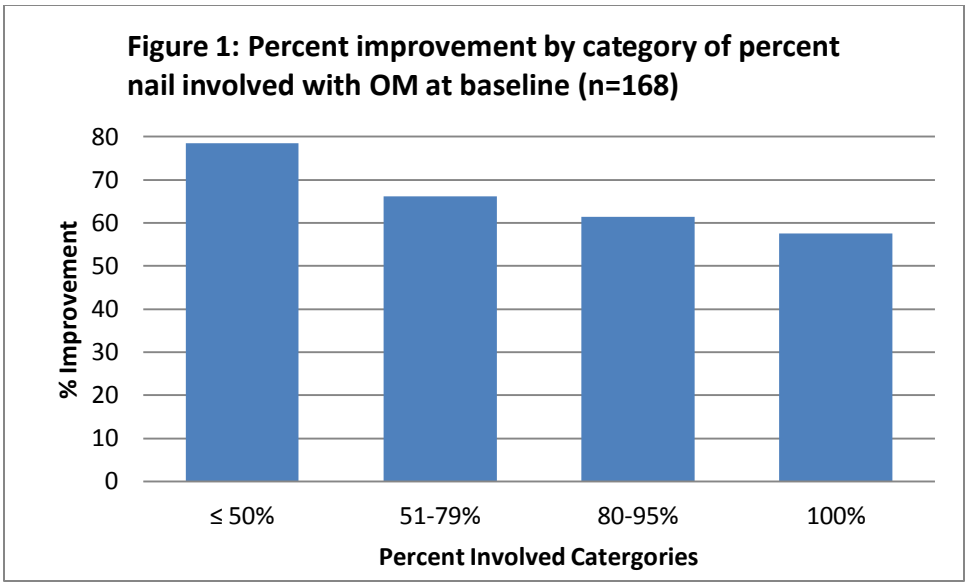
Percent of nail involved	Number of nails
≤ 50%	**32**
51-79%	**25**
80-91%	**34**
100%	**77**

The average percent improvement for all 168 toes were 63.58%, which has reported a statistically significant difference at $p<0.0001$. Variables including gender and foot involvement did not demonstrate any differences regarding the percent improvement of the nail (Table 2).

Table 2: Average percent baseline and endpoint nail involved and percent improvement in clear nail (n=168)

Group	Average Baseline % involved nail	Average Endpoint % Involved Nail	% Improvement in Nail Clarity
All toes/subjects	81.15%	31.32%	63.58%
Male subjects	82.05%	34.41%	59.12%
Female subjects	80.71%	29.78%	65.82%
Right toes	82.67%	33.33%	62.53%
Left toes	79.41%	29.00%	64.80%

Toes which had presented with a lower percentage at baseline reported the greatest percentage of clearance. When comparing the overall percent improvement with the baseline percent of involvement, a correlation between a higher baseline % of involvement and lower % improvement is observed. The greatest improvement was observed for toes with ≤50% of involvement at baseline displaying an average percent improvement of 78.51%.

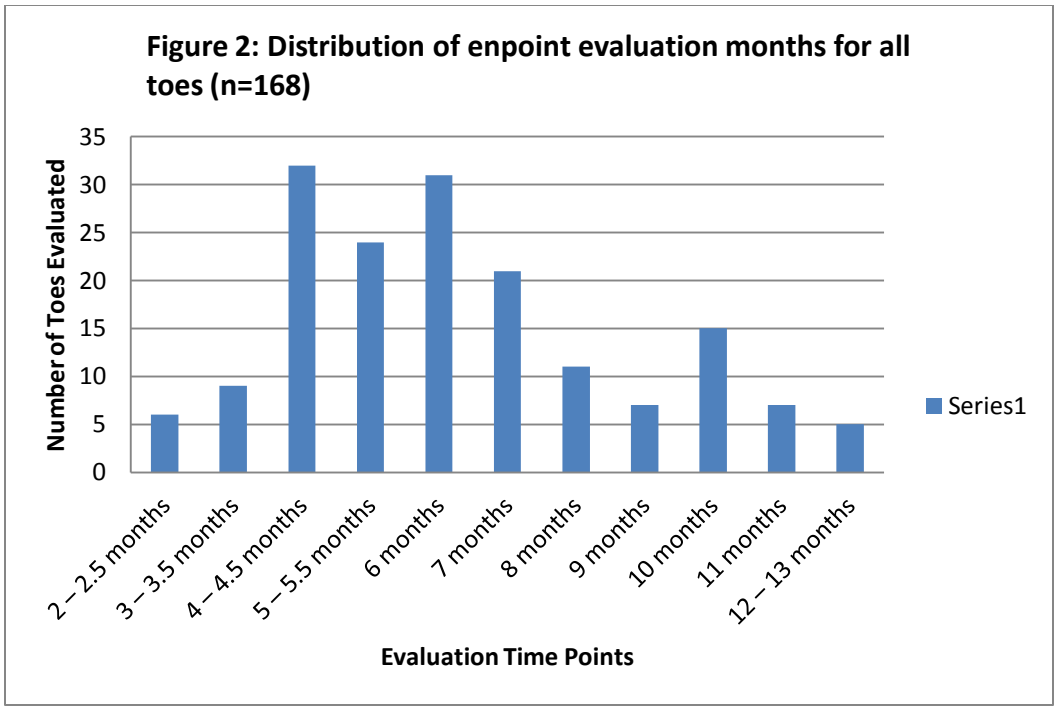


Each percent of involvement category demonstrated a statistically significant percent improvement when comparing the average baseline percent involvement with the average endpoint percent involvement (Table 3).

Table 3: Baseline and endpoint nail involved and percent improvement based on respective percent involved category (n=168)

% nail involved range	*n*	*μa-μb*	*t*	*df*	*p*
<= 50%	32	-31.28	+19.5	31	<0.0001
51% - 79%	25	-46.60	+15.34	24	<0.0001
80% - 95%	34	-52.24	+12.75	33	<0.0001
100%	77	-57.53	+20.46	76	<0.0001

A well-defined post-procedure evaluation phase was not implemented; therefore, post-procedure evaluations varied for individual toes with a reported range of 2 to13 months. For 64.3% of all toes examined, the post-procedure evaluation was conducted between months 4 and 7 (Figure 2). The post-procedure evaluation time points have no defined timeframe; furthermore, the post-procedure evaluation points are independent of the toes' baseline percent of involvement.



Irrespective of the post procedure evaluation endpoint, all toes exhibited an improvement in nail clarity above 50% with a reported range of improvement of 54.34% to 81% (Table 4.). However, no consistent trend was observed concerning the length of duration and reported percent of improvement.

Endpoint month (# months following laser procedure administration)	Average % Improvement in clear nail
2 – 2.5 months	61.30%
3 – 3.5 months	73.89%
4 – 4.5 months	61.63%
5 – 5.5 months	59.19%
6 months	75.98%
7 months	54.34%
8 months	58.13%
9 months	57.14%
10 months	61.12%
11 months	56.90%

12 – 13 months	81.00%

Irrespective of the baseline percent involvement that was reported for toes, no required dates the post-procedure follow-up phase has enabled toes to return at any time following at least two months after the completion of post-procedure administration phase. For toes demonstrating 100% involvement at baseline an argument could be made that a two month follow-up visit would not be a sufficient timeframe to allow for improvement. Toes with 100% involvement exhibited a broad distribution for the post-procedure evaluation phase (Table 5).

Table 5: Post-procedure evaluation time points for toes with 100% involvement at baseline (n=77)

Endpoint Mth	#toes with 100% involvement at Baseline	% improvement in clear nail at Endpoint
2.5	2	62.50%
3.5	3	61.67%
4	6	47.50%
4.5	1	35%
5	12	52.50%
5.5	1	50%
6	19	68.16%
7	9	42.78%
8	5	54%
9	5	62.50%
10	8	65.63%
11	3	46.67%
12	2	62.50%
13	1	80%

The rate of improvement exhibited no defined trends and was independent of the month the post-procedure evaluation was performed.

Although the percent improvement was greater for nails presenting with a smaller percent of involvement at baseline, the percent improvement for nails with a larger initial involvement required clearance across a larger surface. For instance, a 50% improvement for a nail with 100% percent involvement required a quantifiably larger improvement compared to a nail with ≤50%. Comparison of baseline and post-procedure images shows a more prominent visual change for toes exhibiting a higher degree of involvement at baseline (Figure 3).

Discussion:

These data demonstrate the potential utility of a dual diode low-level laser device for the treatment of OM with variable degrees of percent involvement. The viability of this therapeutic approach is best evidenced by the percent improvement of 61.3% and 73.89% observed in just 2.5 and 3.5 months, respectively. This clinical outcome was also achieved independent of nail debridement and adjunctive therapies. Additionally, no adverse events were reported, indicating the potential of this technology to treat a broader patient population irrespective of comorbidities that customarily would have excluding them from mainstay OM treatments. This investigation was an initial effort to determine whether these defined output parameters of low-level laser could generate an observable improvement for OM. It is understood that further clinical studies with more stringent and better defined clinical parameters are warranted in order to properly elucidate the overall efficacy and safety of this application. The poorly defined post-procedure evaluation phase represents a major limitation of this study. Without having a defined post-procedure follow-up phase, these data fail to show the rate of improvement subsequent to treatment. Furthermore, having had just a single follow-up

visit, the study was unable to trend the percent clearance. Additionally, the variability observed for all toes at baseline prevented this study from determining a reliable overall percent improvement for all 168 toes. By implementing more stringent inclusion and exclusion criteria, future studies will have minimized discrepancy, in turn, producing a more reliable overall percent improvement for enrolled toes. However, it was the intention of this pilot investigation, not to define the overall efficacy and safety of this application; but rather, perform the initial steps towards understand the utility of LLLT with specific output parameters for the treatment of OM.

Preceding clinical studies evaluating laser therapy for the treatment of OM have included the use of more high-powered devices. Landsman et al. (2010) have applied 870- and 930-nm near infrared light delivered with an output intensity of 1.7 W/cm^2. By general definition, this output intensity defines this device as a class IV laser, which depending on the applied treatment time, can achieve photothermal energy dosage. Therefore, this treatment requires the observation of temperature with a thermal measuring device. The study has reported 17 of the 26 treated toes have demonstrated an improvement >/- 3.0 mm at 180 days post-treatment. Improvements of 1- <2 mm and <1.0 mm have been reported for 5 and 4 toes, respectively. Using an independent review panel, Landsman et al. (2010) have reported that of the treated toes that 18 of the 26 demonstrated a slight or moderate improvement at day 180. The independent review panel has reported that a single toe demonstrated complete clarity. This study demonstrates the potential utility of laser therapy with different output parameters, and having completed comparative studies in the future, we will be able to determine the most effective output parameter concerning laser therapy for the treatment OM.

Although the exact mechanism has not been fully elucidated, several studies have been able to demonstrate the subtle therapeutic approach of LLLT. The first law of photochemistry states that a photoacceptor molecule, which is a molecular structure capable of absorbing light energy, must be present in order to modulate a cell's pathology. Containing numerous photoreceptors, eukaryotic cells are inducible following laser stimulation when delivered at a specific wavelength. Cytochrome c oxidase, a terminal enzyme of the electron transport chain, has been identified as a potential photoacceptor structure. Defined as a chromophore structure, cytochrome c oxidase has been reported to enter a hyperactive state following laser exposure. As a result, the bioenergetic landscape of the treated tissue shifts to a more conducive state for adenosine triphosphate synthesis. Consequentially, numerous biochemical cascades regulated by the biocatalyst ATP and the intracellular redox are directly affected, in turn, modulating the cell's behavior and function. The synthesis of ATP inexorably accounts for the production of reactive oxygen species since the terminal electron acceptor is oxygen. It has been proposed that the production of high-reactive superoxides results in the biochemical degradation of the protective fungi membrane, a process referred to as lipid peroxidation. Increased membrane permeability will affect the osmotic gradient for the fungi perhaps modulating cell homeostasis. Photoabsorption is dependent on the wavelength that is delivered; therefore, selection of the correct wavelength is important for determining the type of anticipated response. Photoexcitation occurs subsequent to photon absorption of electrons surrounding an atom's nucleus. When photonic energy is absorbed by electrons they are propelled from a ground state (lower energy state) to an excited energy state. As the wavelength changes, so too does the

state of electron excitation. Specifically, as the wavelength is lowered, the energy carried within individual photon increases. Comparing a 400-nm wavelength with a 600-nm wavelength, the 400-nm wavelength will demonstrate a higher quantity of energy per photon, measured in units of electron volts (eV). If the wavelength were decreased below 400-nm, the energy per photon can possess significant deleterious effects because of the increased potential of ionization, which is the absorption of energy so great that the electrostatic force between the electron and nucleus is overcome. Therefore, the application of a 405-nm laser is believed to provide the highest, yet safest, quantity of photonic energy in order to maximize photoacceptor stimulation.

To enhance endogenous biochemical mechanisms, the 635-nm wavelength was combined with 405-nm with the hope of promoting a photochemical response, in turn, simulating the function of immunological cells including resident macrophage and neutrophils. Dolgushin et al. (2010) have reported improved bactericidal activity of neutrophilic granulocytes by upregulating the percent of neutrophils forming extracellular neutrophil traps [4]. Buduli et al. (2004) have evaluated the influence LLLT has on various immunologic parameters for patients with *Helicobacter pylori (H. pylori)* [5]. The authors have reported that low-level laser therapy increased humoral immunity and phagocytic activity of neutrophils, reducing the colonization of *H. pylori* [5]. Regarding macrophage stimulation, Dube et al. (2003) have reported increased phagocytic activity of macrophages in a dose dependent manner [6].

These results demonstrated that the combination of an anti-microbial and photostimulatory low-level laser device was capable of generating both a meaningful and significant improvement in OM.