Erchonia Lunula Laser Therapy (Cold Laser) in the Treatment Onychomycosis.

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Abstract

Onychomycosis is a common disorder of the nails and affects all ages. Treatment modalities include oral and topical antifungals, surgical treatment and or a combined use of these therapies. Cure rates remain low with relatively high relapse rates seen after successful treatment. The purpose of this study is the evaluation of the treatment of onychomycosis using the Erchonia Lunula cold. The study has to date been carried out on 323 patients both male and female with an average age of 40. The laser treatment, in this protocol, consists of 4 treatments at weekly interval. This is a laser that combines two different wavelengths of laser light - one at 405 nm for direct fungicidal activity and one at 635 nm to stimulate a natural immune response - to provide effective clearing of the nail bed. Unlike other lasers used for the treatment of this condition, the Erchonia Lunula laser is reported to cause no pain and no temperature change in the area exposed to the lights. The follow up intervals are 12, 24,36, and 48, further data will be collected in the none treatment phase at 52, 64 and 76 weeks and published at conclusion, as it is the aim of the researchers to observe the nail up to seventy six weeks to ascertain the long term efficacy of the treatment. In the treatment to date there has been 36 reports of side effects and the majority 93% of patients are happy with the treatment. It is the primary aim of this study to present laser as an effective treatment for onychomycosis with a good evidence base.

Key words: low-level laser therapy, photochemistry, onychomycosis

Introduction

Onychomycosis (OM) is a chronic fungal infection of the nail plate, nail bed, or both, and has been estimated to affect 3-10% of the general population, with the incidence rising sharply, to nearly 30%, in patients over age 60 (Welsh, Vera-Cabrera, and Welsh 2010). Although OM is categorized into five different types, distal lateral subungual onychomycosis (DLSO) is the most common clinical presentation (Weinberg et al 2003). It has been reported that the instance of OM is as high as 90% in a 4,096 patient cohort (Weinberg at al 2003, Romano, Giamni and Difono 2000). If left untreated, DLSO can lead to subungual hyperkeratosis, which, in turn, can lead to substantial keratinous debris and induce both nail pressure and pain (Mahoney, Bennett and Olsen 2003). Additionally, OM has been reported to predispose patients to more serious

comorbidities, such as bacterial infection, foot ulceration, cellulitis, thrombophlebitis, and gangrene (Thomas et al 2010, Taylor and Boyle 2003, Gupta and Shear 2000, Roberts, Elewski 2000, Levy 1997). Successful treatment of OM has been hindered by inappropriate therapeutic solutions, with poor clinical outcomes, high rates of recurrence, low patient compliance, or risks of adverse events (Thomas et al 2010). Low-level laser therapy (LLLT), which adheres to the tenets of photochemistry, is emerging as an alternative treatment which may have positive clinical outcomes in the treatment of OM, given effects of light on intracellular reactions (Ying-Ying et al 2009, Karu 2007, Lubart et al 2005, Karu and Afanasyeva 1995). Laser's influence on cell behaviour follows modulation of the cell's bioenergetics: specifically, upregulation of adenosine triphosphate and reactive oxygen species (ROS) synthesis (Ying-Ying 2009). This mechanism can be likened to the agonist effect of a drug, which describes the use of a certain molecule to start a secondary cascade. Laser therapy uses photonic energy to modulate secondary cellular reactions. Supported by nearly four decades of clinical research, LLLT has led to favorable clinical outcomes without serious adverse events (Karu 2007).

In the treatment of OM, LLLT, when delivered with specific parameters, has been shown to decrease dermatophyte colonisation and to strengthen the function of phagocytes, such as neutrophils and macrophages (Dolgushin, Markova and Gizinger 2010, Morgan and Rashid 2009, Dolgushin, and Gizinger 2008, Hemvani, Chitnis and Bhagwanani 2005, Dube et al 2003). Accordingly, stimulation of the body's endogenous defence systems and antimicrobial effects suggest LLLT as a potentially suitable treatment for OM. This study, evaluates patients presenting with OM to examine the efficacy of a dual-wavelength laser device.

Materials and Methods:

A prospective, non-randomized, non-controlled study was conducted from February 2011 to February 2012. Participants who presented with typical clinical patterns of OM of the great toenail and lesser digits were evaluated and mycology samples taken and cultured. All participants with a positive mycology qualified for and were enrolled in the study. In total, 323 subjects were qualified and enrolled and 2320 toes were subject to treatment. As all participants had a positive mycology and no placebo was used a control group was not required. One hundred and fifty patients were chosen at random for mycology testing one week post first treatment. It is worth noting that core sampling showed negative mycology in all patients screened

Inclusion criteria:

- OM in at least one great toenail with an involvement of at least 10%. Spikes of disease extending to the matrix of the effected great toenail and or digits nails.
- Proximal Subungual Onychomycosis (PSO).
- Distal Lateral Subungual Onychomycosis (DLSO).
- White Superficial Onychomycosis (WSO).
- Endonyx Onychomycosis (EO).
- Candidal Onychomycosis (CO).
- Participants willing and able to refrain from the use of nail cosmetics, such as clear and/or coloured nail lacquers throughout the active treatment stage of the study.
- Participants had not used a clinical nail treatment in the past 3 months.
- Study was restricted to patients aged 18 years or older.

Exclusion criteria:

- Participants who have used oral antifungal medicines within 3 months prior to the administration of the first laser treatment.
- Participants who have used nail lacquers in the past three months.
- Those who are unable to abstain from the use of nail cosmetics.
- Pregnant or receiving fertility treatments.
- Metal surgical pins or plates below the knee.
- Chronic plantar (moccasin) tinea pedis,
- Trauma to the affected toenail, or any toenail to be treated.
- Cancer and/or treatment for any type of cancer within the last 6 months,
- History of uncontrolled diabetes mellitus.
- Other exclusions are nevoid subungual formation, psoriasis of the nail plate, atopic dermatitis and lichen planus.

Subject Demographics

In total, 323 subjects were enrolled with percent nail infected with onychomycosis between 12% and 100%. Based on initial percent nail involvement, subjects were allocated into five distinct categories. For measurement methods and calculations please refer to appendix 1.

Category of % Nail Infected With Onychomycosis at Baseline	n
< 20%	81
21% - 40%	71
41% - 60%	110
61-80%	41
> 80%	20

Table 1. Subject allocation based on baseline percent disease involvement

Patients were not compensated financially to participate in the clinical study. Furthermore, patients were recruited from the clinicians existing patient pool who, at one time, actively sought treatment of OM. The study was approved by the Institute of Chiropodists and Podiatrists Ethical Review Board.

Outcome measured included the change in the percent of nail infection. Pre-procedure (baseline) images were taken (as outlined) of the infected nail, which were then compared with procedure and post-procedure images. Toes were evaluated at five separate time points: baseline and week 12, 24, 36, and 48.at which time results were produced. Participants are invited to take part in further follow ups and 52, 64 and 76 weeks post treatment in order to chart any re infection in this 18 month study. Nail assessments were made by the study's principle investigators and measurements were calculated independently and converted to a percentage in keeping with the methods outlined in appendix 1.

Device Intervention

Subjects received treatment with a dual-diode low-level laser device that emitted two independent, rotating line-generated coherent beams, each emitting a separate and discrete wavelength (405 nm and 635 nm) with a total output intensity of ~32 mW (Erchonia Lunula Laser. Specifications in appendix 2).

Treatment Administration

The treatment administration phase consisted of four independent 12-min treatments separated by 7 days ± 1 day.
All patients were treated as outlined below at each of their four visits.

1. The foot to be treated was cleansed using a clinell wipe.
2. Nails were clipped and reduced using a bur where necessary.
3. The foot was cleansed to remove any debris and dust.
4. The forefoot was photographed using a high resolution digital camera.
5. Infected shoes, socks and hosiery were sterilised.
6. Laser administered in the therapy unit, these are programmed to deliver constant therapy for 12 minutes and then turn off. All toes of the infected extremity, regardless of clinical presentation of OM, received equal exposure to the emitted laser energy.

It is important to note that no chemicals were used to pre soften thickened nails as the researchers wanted to remove any possibility of interference from outside sources.

Data Analysis

The study is an 18 month study with evaluations planned at Baseline (pre-treatment); 12 weeks; 24 weeks; 36 weeks; 48 weeks; 52 weeks; 64 weeks; and 76 weeks post-procedure. The current analysis is based on measurements recorded at Baseline; 12 weeks, 36 weeks and 48 weeks post-procedure. A repeated-measures ANOVA was used to assess the five independent sample means along with mean and standard deviations. Calculation of percent nail clearance at each evaluation point showed the changes in mean percent nail clarity to be statistically significant across the four correlated samples ($F = 199.2$; $p < 0.0001$).

Results

All study participants ($n = 323$) were categorized into five groups based on their initial percent of nail involved. Compared with baseline, statistically significant mean change in the percent of nail involved was observed at weeks 12, 36 and 48 for each baseline category. Table 2 below shows the mean and standard deviation % of nail onychomycosis inclusion at each of the 4

evaluation points of Baseline, 12 Weeks, 36 Weeks and 48 Weeks Post-Procedure for nails within each of the 5 categories of % Baseline inclusion and for all subjects combined.

		% NAIL ONYCHOMYCOSIS INCLUSION			
BASELINE CATEGORY % ONYCHOMYCOSIS INCLUSION		**Baseline**	**12 Weeks Post-Procedure**	**36 Weeks Post-Procedure**	**48 Weeks Post-Procedure**
< 20%	Mean	18.12%	3.52%	0%	0%
	SD	*2.13*	*2.90*	*0*	*0*
21% - 40%	Mean	34.94%	10.42%	1.20%	0.18%
	SD	*5.34*	*6.56*	*3.30*	*0.80*
41% - 60%	Mean	54.29%	14.59%	1.92%	0.40%
	SD	*4.80*	*7.08*	*2.69*	*0.97*
61% - 80%	Mean	75.51%	32.61%	12.17%	6.98%
	SD	*5.49*	*11.26*	*9.58*	*9.62*
> 80%	Mean	97.40%	57.05%	30.9%	17.75%
	SD	*3.49*	*10.13*	*6.87*	*10.77*
TOTAL	***Mean***	**46.33%**	**15.81%**	**4.37%**	**2.16%**
	SD	***23.25***	***15.39***	***8.92***	***6.32***

Table 2: *% Nail Onychomycosis Inclusion across Study Evaluation by % Baseline Inclusion Category*

Analysis of four correlated samples found the changes in mean % nail onychomycosis inclusion across the three evaluation points of Baseline, 12, 36 and 48 Weeks Post-Procedure to be statistically significant for all toes combined, and for toes within each of the 5 individual categories of Baseline % nail onychomycosis inclusion. For each individual baseline category, a statistically significant mean change was observed from baseline at weeks 12, 36 and 48.

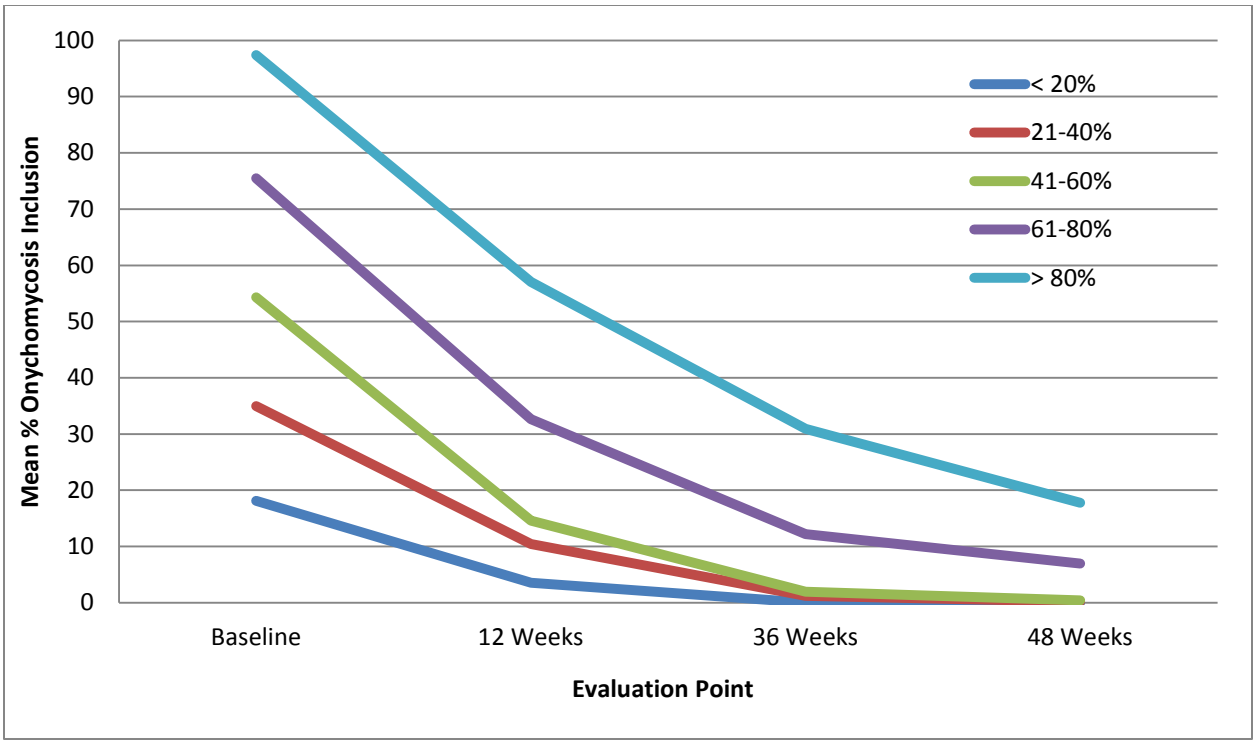


Chart 1: *% Nail Onychomycosis Inclusion by Evaluation Visit by Baseline % Onychomycosis Inclusion Category*

As can be seen in Table 2 and Chart 1 above, regardless of the extent (%) of nail onychomycosis involvement at baseline, by 48 weeks post-procedure, almost all nails with 80% or less onychomycosis involvement at baseline were completely or almost completely disease free. The highest incidence of residual onychomycosis at 48 weeks post-procedure occurred for toes in the > 80% Baseline onychomycosis inclusion category, although the mean % disease involvement did decrease from 97.4% at baseline evaluation to less than a one-fifth of this baseline % to 17.75% at 48 weeks post-procedure evaluation. It is not surprising that this category wherein the toes were almost completely disease involved at baseline showed more residual disease involvement at 48 weeks post-procedure, as given the relative greater severity of baseline inclusion, it would be anticipated and expected that it would take longer for the nail to grow and clear regardless of the type or efficacy of treatment intervention. In fact, the finding that less than 18% of each toe on average remained disease involved at 48 weeks post-procedure is quite significant.

CHANGE IN % NAIL ONYCHOMYCOSIS INCLUSION ACROSS EVALUATION POINTS

ANOVA analysis for 4 correlated samples found the changes in mean % nail onychomycosis inclusion across the 4 evaluation points of Baseline, 12 Weeks, 36 Weeks and 48 Weeks Post-Procedure to be statistically significant for all toes combined, and for toes within each of the 5 individual categories of Baseline % nail onychomycosis inclusion, as follows.

- *All Toes Combined* (n=323)

  F=1442.27; p<0.0001

Subsequent Tukey HSD Test analysis found the changes between each of the 4 evaluation points for all toes combined to be statistically significant:

- ✓ Baseline to 12 Weeks Post-Procedure: p<0.01
- ✓ Baseline to 36 Weeks Post-Procedure: p<0.01
- ✓ Baseline to 48 Weeks Post-Procedure: p<0.01
- ✓ 12 Weeks to 36 Weeks Post-Procedure: p<0.01
- ✓ 12 Weeks to 48 Weeks Post-Procedure: p<0.01
- ✓ 36 Weeks to 48 Weeks Post-Procedure: p<0.05

- *< 20% Baseline % Nail Onychomycosis Inclusion* (n=81)

  F=2045.42; p<0.0001

Subsequent Tukey HSD Test analysis found the changes between each of the 4 evaluation points for toes with < 20% onychomycosis inclusion at Baseline to be statistically significant, at p<0.01:

- ✓ Baseline to 12 Weeks Post-Procedure
- ✓ Baseline to 36 Weeks post-Procedure

- ✓ Baseline to 48 Weeks Post-Procedure
- ✓ 12 Weeks to 36 Weeks Post-Procedure
- ✓ 12 Weeks to 48 Weeks Post-Procedure

➢ *21% - 40% Baseline % Nail Onychomycosis Inclusion* (n=71)

F=1397.05; p<0.0001

Subsequent Tukey HSD Test analysis found the changes between each of the 4 evaluation points for toes with 21% - 40% onychomycosis inclusion at Baseline to be statistically significant, at p<0.01:

- ✓ Baseline to 12 Weeks Post-Procedure
- ✓ Baseline to 36 Weeks post-Procedure
- ✓ Baseline to 48 Weeks Post-Procedure
- ✓ 12 Weeks to 36 Weeks Post-Procedure
- ✓ 12 Weeks to 48 Weeks Post-Procedure

➢ *41% - 60% Baseline % Nail Onychomycosis Inclusion* (n=110)

F=4718.06; p<0.0001

Subsequent Tukey HSD Test analysis found the changes between each of the 4 evaluation points for toes with 41% - 60% onychomycosis inclusion at Baseline to be statistically significant, at p<0.01:

- ✓ Baseline to 12 Weeks Post-Procedure: p<0.01
- ✓ Baseline to 36 Weeks Post-Procedure: p<0.01

- ✓ Baseline to 48 Weeks Post-Procedure: $p<0.01$
- ✓ 12 Weeks to 36 Weeks Post-Procedure: $p<0.01$
- ✓ 12 Weeks to 48 Weeks Post-Procedure: $p<0.01$
- ✓ 36 Weeks to 48 Weeks Post-Procedure: $p<0.05$

- *61% - 80% Baseline % Nail Onychomycosis Inclusion* (n=41)

  $F=1220.32$; $p<0.0001$

Subsequent Tukey HSD Test analysis found the changes between each of the 4 evaluation points for toes with 61% - 80% onychomycosis inclusion at Baseline to be statistically significant, at $p<0.01$:

- ✓ Baseline to 12 Weeks Post-Procedure
- ✓ Baseline to 36 Weeks post-Procedure
- ✓ Baseline to 48 Weeks Post-Procedure
- ✓ 12 Weeks to 36 Weeks Post-Procedure
- ✓ 12 Weeks to 48 Weeks Post-Procedure
- ✓ 36 Weeks to 48 Weeks Post-Procedure

- *> 80% Baseline % Nail Onychomycosis Inclusion* (n=20)

  $F=619.93$; $p<0.0001$

Subsequent Tukey HSD Test analysis found the changes between each of the 4 evaluation points for toes with 61% - 80% onychomycosis inclusion at Baseline to be statistically significant, at $p<0.01$:

- ✓ Baseline to 12 Weeks Post-Procedure

- ✓ Baseline to 36 Weeks post-Procedure
- ✓ Baseline to 48 Weeks Post-Procedure
- ✓ 12 Weeks to 36 Weeks Post-Procedure
- ✓ 12 Weeks to 48 Weeks Post-Procedure
- ✓ 36 Weeks to 48 Weeks Post-Procedure

Therefore, the mean % nail onychomycosis inclusion decreased progressively and significantly across the current 48 Week Post-Procedure evaluation phase, indicating progressive ongoing positive treatment effect of the Erchonia Lunula laser.

COMPLETE NAIL CLEARANCE AT 48 WEEKS POST-PROCEDURE

Table 3 below shows the number and percentage of toes that attained complete nail clearance (0% nail onychomycosis inclusion), and 5% or less nail onychomycosis inclusion, at 48 weeks post-procedure for all study toes combined and within each of the 4 individual Baseline % onychomycosis inclusion categories.

Table 3: *Toes Attaining Complete and Toes Attaining 95% or Greater Clearance at 48 Weeks Post-Procedure*

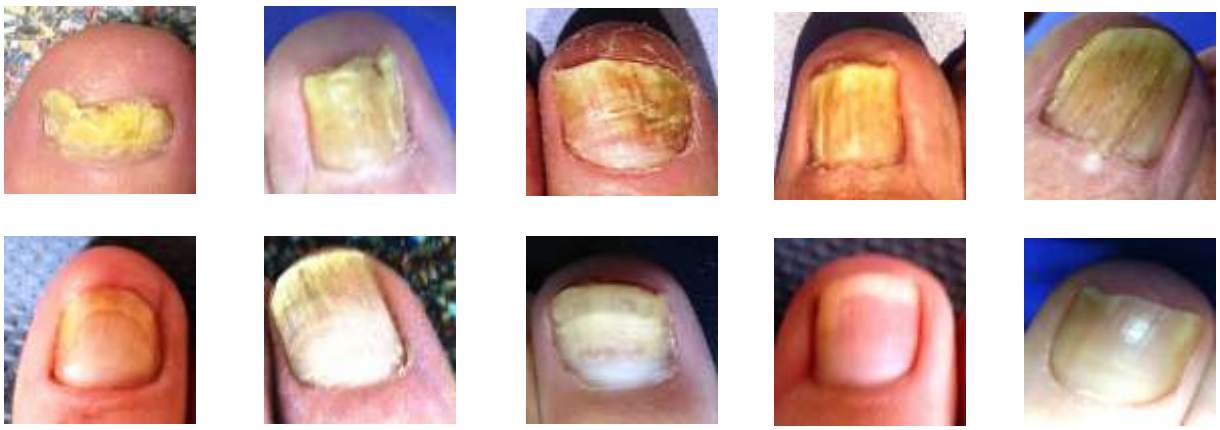
	Nails With Complete Clearance at 48 Weeks		**Nails With 95%-99% Clearance at 48 Weeks**	
Baseline % Onychomycosis Inclusion Category	**n**	**%**	**n**	**%**
< 20% (n=81)	81	100%	81	100%
21% - 40% (n=71)	66	93%	71	100%
41% - 60% (n=110)	91	83%	110	100%
61-80% (n=41)	13	32%	27	66%
> 80% (n=20)	1	5%	1	5%
ALL TOES (n=303)*	**251**	**83%**	**289**	**95%**

** Does not include toes in >80% category*

Overall, more than 8 out of every 10 toes (83%) attained complete clearance of onychomycosis at 48 weeks post-procedure. Not surprisingly, the greatest incidence of complete clearance was attained for toes with the least percentage of Baseline nail onychomycosis inclusion (< 20%), with all 81 (100%) of those toes attaining 100% clearance. Likewise, the incidence of complete nail clearance decreased progressively across the categories of increasing % baseline nail onychomycosis inclusion, from 93% of nails showing complete clearance at about 11 months post-procedure for toes with 21% - 40% Baseline onychomycosis inclusion, through 83% of nails showing complete clearance at 48 weeks for toes with 41% - 60% Baseline onychomycosis inclusion, through 32% of nails showing complete clearance at 48 weeks for toes with 61% - 80% Baseline inclusion to 5% of nails (0%) showing complete clearance at 48 weeks for toes with > 80% Baseline inclusion.

When evaluating toes that demonstrated 95%-99% nail clearance at 48 weeks post-procedure, a 100% rate was attained for toes in each of the 3 Baseline nail onychomycosis inclusion categories of < 20%, 21%-40% and 41%-60%. Sixty-six per cent (66%) of toes in the 61%-80% Baseline nail onychomycosis inclusion category and 5% of toes in the >80% Baseline nail onychomycosis inclusion category attained 95%-99% nail clearance.

Again, this finding is not surprising given that greater degree of disease involvement will naturally take longer to demonstrate complete clearance regardless of the type or effectiveness of treatment intervention.



The above plates show the before treatment (top) and the below plates show 48 post treatment

Discussion

Dermatophytes cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinised material. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Invasion does elicit a host response ranging from mild to severe. Acid protenases, elastase, keratinase and other proteinases reportedly act as virulence factors (Rosenberg and Gallin 1999).

Dermatophytes are transmitted by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in clothing, combs, hair brushes, theatre seats, caps, furniture, bed linens, shoes, socks, towels, hotel rugs, sauna, bathhouse, and locker room floors (Ajello and Getz 1954). Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns, excessive temperature and humidity.

Increasingly Onychomycosis is being viewed as a more cosmetic problem as people become ever more conscious of their appearance. Fungi from the nails may happen before secondary bacterial infections such as cellulitis, idiopathic reactions and chronic urticarial. Infected toenails may act as a reservoir for fungi, facilitations their transmission to other parts of the body and potentially to other people.

Clinical diagnosis of Onychomycosis is based on physical examination, microscopy and culture of nail specimens. Factors such as diabetes, hyperhidrosis, nail trauma, poor peripheral circulation; can contribute to the condition. Differential diagnosis for onychomycosis, as mentioned earlier, should be considered so as to allow the clinician to choose the most appropriate treatment.

It has been found to date, in this study, that 4 treatments for nails up to 60% inclusion has a satisfactory outcome and that nails with over 60% benefit from further pain free treatments.

The two discrete laser light wavelengths used here have been reported to cause specific biological outcomes that are believed to provide a multifaceted treatment for DLSO. First, 635 nm (within the red visible spectrum) has been shown to activate PI3 kinase / eNOS signaling pathways, and to induce endothelial cell migration and neovascularisation (Schindl et al 1999, Lim et al 2011). Furthermore, red light has been shown to improve phagocyte function and to induce a respiratory burst in neutrophils (Duan et al 2001). Conversely, 405 nm has been demonstrated to have an antimicrobial effect by upregulating the production of ROS, leading to the generation of hydrogen peroxide, hypochlorous acid, and hydroxyl radicals (Lavi et al 2012). When applied concurrently, the combined antimicrobial and biostimulative effects appear to

provide a therapeutically beneficial combination, as demonstrated by the mean percent changes in clarity.

A potential phototarget for the 405 nm wavelength is also a system responsible for catalyzing the generation of ROS, nicotinamide adenine dinucleotide phosphate oxidase (NOX) (Lavi et al 2012). NOX transfers electrons from cytosolic NADPH to flavin adenine dinucleotide (FAD), then to extracellular molecular oxygen to generate superoxide (Streeter et al 2012, Lamberth 2004). The third and fifth transmembrane domains of NOX bind two prosthetic heme groups that shuttle electrons from FAD to oxygen (Streeter et al 2012, Lamberth 2004). It has been suggested that the prosthetic heme, which has been recognized as a photosensitizer, responds to the delivery of blue light. Stimulation of NOX could potentially provide two benefits: first, phagocytes are activated, and second, dermatophytes are susceptible to the toxic effects of ROS. Furthermore, squalene epoxidase, the therapeutic target for numerous antifungal medications, depends on the presence of NADPH or NADH and uses FAD to shuttle electrons from NADPH cytochrome P450 reductase. Loosely binding with FAD, SE may be subject to functional aberrations after light exposure (Ono and Bloch 1975).

Conclusion

The data coupled to-date, with the absence of adverse events, substantiated LLLT as a safe and effective treatment for OM. Furthermore, the data demonstrate that LLLT is effective at treating varying degrees of OM. In more severe cases of OM, with an accumulation of keratinous debris, it might be expected that light attenuation would dampen the clinical effect, with active debridement this has been seen not to be the case. Nevertheless, the successful treatment of nails with an initial percent involvement 100% demonstrates the laser's ability to permeate down to the nail bed. However, to understand the full utility of this procedure, further

follow-up data is being collected to evaluate the rate of reoccurrence and or reinfection. Additionally, fungal cultures were obtained and evaluated during the study that along with the visual improvement in nail clarity demonstrate the benefits of this cold laser treatment in destroying Dermatophytes. Clinical success is defined by the absence of clinical signs or the presence of negative nail culture, and not necessarily both (Scher et al 2007). Nevertheless, future studies evaluating LLLT will diagnose OM using microscopy and measure success based on clinical and mycological observations

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Appendix 1

In order to assess and measure the amount of nail included high resolution digital photographs were take using a jig into which the patient foot was placed and on which a camera was mounted. A scale measure marked Left and Right is fixed to the jig in order to have an idea of scale. Once the photographs were processed they were independently measured using a micrometer measuring from the nail fold to the base of the fungus. Where irregular lines or included area were found, triangulation was used to calculate the area involved.

Photos of camera equipment and jig to be added