photonics

MDPI

Article

The Efficacy of Phototherapy for the Treatment of Onychomycosis: An Observational Study

Nadia Dembskey and Heidi Abrahamse *

Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, South Africa; nadia.dembskey@gmail.com

* Correspondence: habrahamse@uj.ac.za; Tel.: +27-11-559-6550

Abstract: (1) Background: Onychomycosis accounts for 50% of nail pathologies and is a therapeutic challenge due to an increase in resistance to antifungal agents. This study aimed to explore the effectiveness of 1064 nm diode laser irradiation for the treatment of Onychomycosis and establish a new set of laser parameters for effective and safe treatment; (2) Methods: An exploratory, single-blinded study was conducted on forty-five patients with toenail Onychomycosis. Digital images and nail clippings were taken for Periodic Acid-Schiff (PAS) staining and fungal microscopy and culture (MC&S). Group 1 received 5% topical Amorolfine lacquer to apply to affected nails. Group 2 received 1064 nm diode laser treatment at 10 mW/s, hallux 790 J/cm^2 and lesser digits 390 J/cm^2 (standard treatment). Group 3 received 1064 nm diode laser treatment at 10 mW/s, hallux 1 100 J/cm^2 and lesser digits 500 J/cm^2 (new treatment parameters). After laser treatment, nail temperatures were taken with a surface thermometer; (3) Results: PAS staining was more sensitive in identifying Onychomycosis (91.1%), compared to Fungal Microscopy (44.4%). Comparing treatment requirements over a period of 24 weeks, there was a statistical significance, $p \leq 0.01$ (**), for standard laser treatment and, $p \leq 0.001$ (***), for new laser parameter treatment, indicating treatment needed over time decreased. No adverse effects were noted with new laser therapy. An 86.7% visual improvement was noted in Group 3 after 24 weeks; (4) Conclusions: Phototherapy, or photo thermolysis, was the best treatment option for Onychomycosis. A new protocol for the standardization of laser irradiation with the possible inclusion into the Scoring Clinical Index for Onychomycosis treatment plan, was proposed.

Keywords: Onychomycosis; phototherapy; Scoring Clinical Index for Onychomycosis

Citation: Dembskey, N.; Abrahamse, H. The Efficacy of Phototherapy for the Treatment of Onychomycosis: An Observational Study. *Photonics* **2021**, *8*, 350. https://doi.org/10.3390/photonics8090350

Academic Editors: Andrea Amaroli, Nasim Chini-forush and Kinga Grzech-Leśniak

Received: 20 July 2021
Accepted: 21 August 2021
Published: 25 August 2021

1. Introduction

Onychomycosis (OM), a chronic fungal infection of the toenails, is a common condition that accounts for 50% of all nail pathologies [1–7]. Currently, one-third of the world population has this condition, and is of great concern [8,9]. The term OM is derived from the Greek word 'onyx' which means a nail and 'mykes' which denotes a fungus [10]. It's primarily caused by dermatophytes but can also be caused by yeasts and non-dermatophyte molds [5,7,8,11–13]. Previously regarded as contaminants, yeasts and some moulds are now increasingly recognised as pathogens [10]. The most noticeable among these are *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Candida krusei* (yeasts) and *Aspergillus fumigates* (mould) [5,11,14,15]. These moldy fungi are especially difficult to cure in OM using standard treatment modalities as they focus on dermatophyte organisms [16,17]. Also, the clinical presentation of OM should be taken into consideration when therapeutic decisions are being made [18]. Table 1 details the clinical picture of OM.

This provides an outline for diagnosis and expected response to treatment. It can also predict the possible prognostic outcomes [20].

1.1. The Scoring Clinical Index for OM (SCIO)

The Scoring Clinical Index for OM (SCIO) attempts to present the severity of OM as a composite score, and aims to identify the type of OM, the area and thickness of nail

involvement, the age of the patient and the location of the digit affected. It is calculated using the clinical index component and growth component. The higher the SCIO, the more severe the OM, which in turn requires a higher dosage and more prolonged treatment [21]. The treatment dosage is for oral and topical antifungals only; laser treatment was a novel treatment in this study with a SCIO attached. An electronic version of the SCIO is available at http://www.onychoindex.com; accessed on 2 July 2021. Table 2 describes the current proposed treatment guidelines according to the SCIO.

Table 1. Clinical picture of Onychomycosis [8,11,19,20].

Classification	Description	Causative Organism
Distal Subungual Onychomycosis (DSO)	• Distal free edge (hyponychium) of nail and spreads to nail plate and bed • Hyperkeratotic debris accumulate and result in onycholysis • Nails become dystrophic • Turn yellow-white or brown-black • Can spread proximally causing linear spikes • Associated with paronychia • Mode of Infection: Through break in skin at the distal under surface of nail	• *Trichophyton rubrum* • *Trichophyton mentagrophytes* • *Epidermophyton floccosum* • *Candida albicans* • *Fusarium species* • *Scopulariopsis brevicaulis* • *Scytalidium species*
Lateral and Distolateral Subungual Onychomycosis (DLSO)	• Continuation from DSO • Pathogen migrates from hyponychium at side proximally • Hyperkeratotic debris accumulate and result in onycholysis • Brownish-yellow • Can spread proximally causing linear spikes • Associated with paronychia • **Mode of Infection**: Through break in skin at the distal under surface of nail	• *Trichophyton rubrum* • *Trichophyton mentagrophytes* • *Epidermophyton floccosum* • *Candida albicans* • *Fusarium species* • *Scopulariopsis brevicaulis* • *Scytalidium species*
Proximal Subungual Onychomycosis (PSO)	• Rare • Debris accumulate under the eponychium, causing onycholysis and spreads distally • Found in immunosuppressed patients • Hyperkeratotic • White • **Mode of Infection**: Invasion of the proximal nail fold and cuticle. May cause secondary paronychia	• *Trichophyton rubrum* • *Candida albicans* • *Aspergillus species* • *Fusarium species*
White Superficial Onychomycosis (WSO)	• Superficial nail plate infection • White plaque-like layer covers the nail plate • Powder-like patches of transverse striae • **Mode of Infection**: Appear om superficial nail plate. May form a deep penetration of the superficial infection	• *Trichophyton rubrum* • *Trichophyton mentagrophytes* • *Acremonium species* • *Fusarium species* • *Scytalidium species*
Proximal White Subungual Onychomycosis (PWSO)	• Special variant • White discolouration underneath proximal part of nail plate • **Mode of Infection**: Invasion of the proximal nail fold and cuticle	• *Trichophyton rubrum* • *Candida albicans* • *Aspergillus species* • *Fusarium species*

Table 1. *Cont.*

Classification	Description	Causative Organism
Total Dystrophic Onychomycosis (TDO)	• "Glacier nail" • Longitudinal yellow streaks stretching medially and laterally reaching the nail matrix • Complete destruction of the nail from longstanding infection • Nail structure is lost	• Can result from any of the other classes • Most often from severe DSO or DLSO infections

Table 2. Proposed treatment guidelines for Onychomycosis treatment according to Scoring Clinical Index for Onychomycosis values [21].

Scio	Treatment Approach
1–3	• Topical treatment • Remove affected minor parts of nail • Use topical antifungals until healthy nail re-grows
3–6	• Topical treatment with lower success • Depends on nail growth rate • Systemic treatment recommended in slower-growing nails or proximal Onychomycosis types
6–9	• Systemic treatment • Use dosage purported for fingernails: For example: Itraconazole 200 mg BD pulse treatment for 2 months
9–12	• Systemic treatment • Use dosage purported for toenails: For example: Itraconazole 200 mg BD pulse treatment for 3 months
12–16	• Systemic treatment • Use dosage purported for fingernails with any antifungal of the clinicians' choice: For example: Itraconazole 200 mg BD pulse treatment for 4–5 months
16–20	• Combination treatment (systemic and topical) • Adequate keratolytic treatment recommended
20–30	• Consider nail avulsion • Continue with systemic treatment

This may prove an accurate indicator of therapeutic effectiveness, but further clinical studies must be done before definitive claims can be made [22]. As we aimed to identify the effectiveness of laser therapy for OM compared to topical treatment, this may bridge the gap of clinical effectiveness by adding laser therapy as a possible route of treatment, depending on the SCIO.

1.2. Diagnosis

Only 50% of nail pathologies are caused by OM, so adequate diagnosis is essential for treatment [12]. Clinical examination may not always be precise and laboratory testing should be included for accurate diagnosis [20]. Nail clippings and scrapings are the most common sampling methods for suspected OM [11,12,23]. The simplest method for detecting fungi is by way of 20% potassium hydroxide (KOH) preparations. However, it shows only 40–60% sensitivity [5,14]. Fungi can also be grown in culture form, using Sabouraud 2% dextrose agar; however, a 70% sensitivity failure rate is seen, possibly due to

antifungal agent use [7,13]. Recently, histological fungal detection i.e., Periodic Acid-Schiff (PAS) stain, has shown high sensitivity (92%) in the detection of fungal elements; however, specificity is low as there is no indication of fungal genus or species [8,14]. Wilsmann-Theis, et al., writes that to date, the gold standard in the diagnosis of OM remains direct microscopy and culture [23]. They compared the current gold standard in OM diagnosis with histological PAS staining in a large cohort. A total of 631 samples revealed a positive result in at least 1 test. They found that the most sensitive single test for the diagnosis of OM was PAS staining (82%), followed by culture (53%) and direct microscopy (48%). In 64 cases where prediagnostic antimycotic treatment was implemented, PAS staining had the highest sensitivity (88%) in comparison with culture (33%) or direct microscopy (50%) [23]. Although PAS staining is shown to be the single method with the highest sensitivity in terms of detecting fungal hyphae (especially in cases with prior antimycotic therapy), it still has a very low specificity rate [8,14,23]. Kaur, et al., recommends that PAS stain and culture should be done together [10].

1.3. Current Treatment Modalities for OM

Several factors e.g., infecting organism, clinical presentation, severity of infection and co-morbidities should be taken into consideration when treating OM [12,16]. Oral antifungal agents (terbinafine hydrochloride, itraconazole and fluconazole) are the medications of choice for this condition, as either continuous or "pulse" therapies [11,14,24]. Recently, there has been an increase in resistance shown to these medications and various questions remain as to their safety for patients and their potential to cause hepatotoxicity [3,4,9,11,15–17]. Topical antifungal treatments (amorolfine and ciclosporox) are preferred by most patients as they have less serious side effects. These agents can only be administered to those who have OM without matrix involvement and requires extensive patient monitoring [9]. Serious questions also arise on the effectiveness of nail penetration as well as issues relating to increased organism resistance [1,9,11,15,25].

1.4. Phototherapy

Limitations in outcomes in both these antifungal agents have led to the investigation of laser and light as a possible new way of treating OM [3,18,24]. This treatment modality has been in the spotlight since 2010, although mentioned in 1980 [14]. Studies show lasers of near-infrared light range with wavelengths of 780–3000 nm being used, but most commonly the Neodymium Yttrium Aluminium Garnet (Nd: YAG) 1064 nm laser [13,21]. Preliminary studies show good clinical and microscopical cure rates using the Nd: YAG 1064 nm laser in the short term [2,4,12,18,25]. In 2012, the Food and Drug Administration (FDA) approved the use of lasers for the "temporary increase in clear nail in Onychomycosis" [12]. This is based on a cosmetic outcome that differs from the medical efficacy approvals granted to topical and oral antifungal agents and may be partially due to poor published study designs with small study samples. Device-based therapies are promising alternatives for OM treatment, as they can mitigate some of the negative factors associated with treatment failure [26]. Authors described the Laser therapy for OM as based on the "principle of selective photo thermolysis", for which the pathogen absorbs the light energy and converted it into heat. Fungi are considered by the authors [2,4,22,25] sensitive to heat above 55 °C, so the absorption generates photothermal heating of the fungal structures with fungicidal effects. However, heating dermal tissue to above 40 °C produces pain and necrosis [2,4,22,25]. The authors correctly point out that in the literature it is considered necessary to pulsate or keep "low" the levels of radiated energy, in laser treatment, to avoid overheating that is harmful to the patient. Currently, lasers of different wavelengths, pulse duration and spot size are used throughout literature [22,26–30]. In studies found, no standard podiatric treatment was done prior to laser treatment, not enough time was given between treatments in order to see clear nail growth, with limited output, and no clear protocol could be attained [14,31]. With onychomycosis in progress, urgent intervention is required, decided by a doctor, a podiatrist, or in any case a specialist, who must choose

the antifungal drug treatment with topical or systemic action deemed most appropriate for the subject. It must be considered that this treatment is usually long since the nails take from eight, nine months, up to a year to fully regrow. For this reason, the search for alternative treatments to conventional antifungals is useful and necessary. For this purpose, the authors wanted to investigate the effectiveness of the 1064 nm diode laser irradiation for the treatment of OM and establish a new set of laser parameters for effective and safe treatment [32].

2. Materials and Methods

Ethical clearance was granted from the University of Johannesburg Faculty Ethics Committee (REC-01-176-2015) and participating patients gave written consent.

We obtained the use of the 1064 nm Nd: YAG Fox diode laser form 1360 Moderno predgradie, Sofia, Bulgaria, for the two study groups. Wavelength was set to 10 W (regardless of fluence) with a pulse length of 0.1 ms, pulse interval of 0.1 ms and spot size of 2 mm.

Three groups were used in a single-blinded study performed with 45 toenail Onychomycosis patients for 12 weeks of treatment. The investigation techniques used were image acquisition by Periodic Acid-Schiff (PAS) staining on the nail. clippings and fungal microscopy and culture. Of the 3 groups, Group 1 was treated with 5% topical Amorolfine lacquer, Group 2 was subjected to treatment using the 1064 nm diode laser (10 mW/s, hallux 790 J/cm^2, and lesser digits 390 J/cm^2) as standard treatment, while Group 3 received 1064 nm diode laser treatment at 10 mW/s, hallux 1 100 J/cm^2 and lesser digits 500 J/cm^2, come new treatment parameters to investigate. Correctly, at the end of the laser treatment, nail temperatures were measured with a surface thermometer. Really, despite the use of light and laser as treatment of mycosis, was considered in scientific literature since the early '80, as correctly reported by authors, when in 2012, the Food and Drug Administration (FDA) approved the use of lasers for the "Temporary increase in clear nail in Onychomycosis" this decision was based on a cosmetic point of view only and not from the medical efficacy. Table 3 outlines the different laser parameters used for Groups 2 and 3.

Table 3. Laser parameter settings for Group 2 (Standard Laser Treatment) and Group 3 (New Laser Treatment).

Group	Wavelength	Frequency	Pulse Length	Pulse Interval	Aiming Beam	Spot Size
2	10 mW/s	Hallux; 790 J/cm^2	0.1 ms	0.1 ms	Green; 532 nm	2 mm
		Lesser Digits; 390 J/cm^2	0.1 ms	0.1 ms	Green; 532 nm	2 mm
3	10 mW/s	Hallux; 1 100 J/cm^2	0.1 ms	0.1 ms	Green; 532 nm	2 mm
		Lesser Digits; 500 J/cm^2	0.1 ms	0.1 ms	Green; 532 nm	2 mm

At Stage 1 (Week 1), we classified the type of OM and noted this on the Patient Care Form. Afterwards, patients were randomly grouped into three groups (single-blind). Digital images of the toenails were taken at 10 cm distance to establish the severity of fungal infection before treatment. All patients received standard podiatric treatment (cutting and drilling down of nails to 2–3 mm thickness and post-drilling cleaning of nails with 50% Hibitaine solution to get grid of any contaminants [18,33]) before treatment with laser or control. Nail clippings were taken at the site of infection for PAS staining and fungal MC&S to identify fungal elements and isolate the causative pathogen prior to treatment. Clippings were placed into two separate specimen bottles—one that contained formalin to fix the organism (PAS staining); the other in an empty specimen bottle to allow for fungal culture and microscopic analysis [5,34]. Patients received a Numeric Pain Scale form to document their level of discomfort during each treatment, omitting the control group. Group 1 received 5% topical Amorolfine lacquer to apply to affected nails once weekly. Group 2 received 1064 nm diode laser energy treatment in a grid

pattern on each toenail, regardless of infection at 10 mW/s wavelength, which is the visible colour spectrum, hallux 790 J/cm^2 frequency, which is the oscillation frequency of the corresponding electromagnetic wave, or the laser mode, and lesser digits 390 J/cm^2 frequency, pulse length 0.1 ms, pulse interval 0.1 ms, aiming beam green 532 nm, and spot size 2 mm. Group 3 received 1064 nm laser energy treatment in a grid pattern on each toenail, regardless of infection at 10 mW/s wavelength, which is the visible colour spectrum, hallux 1 100 J/cm^2 frequency and lesser digits 500 J/cm^2 frequency, which is the oscillation frequency of the corresponding electromagnetic wave, or the laser mode, pulse length 0.1 ms, pulse interval 0.1 ms, aiming beam green 532 nm, and spot size 2 mm. Immediately after laser treatment within each group, nail temperature reached was taken with a surface thermometer. Specimen bottles with tissue were sent to a private laboratory where diagnostic tests were conducted.

At Stage 2 (Week 12), digital images of the toenails were taken to establish the severity and/or improvement of fungal infection. Standard podiatric treatment was given again. This time, nail scrapings were then taken at the site of the eponychium (or most proximal site of infection) for PAS staining fungal MC&S to identify whether fungal elements and the causative pathogen were still present at the site of new nail growth. All data was recorded on the second Patient Care Form. Patients were again given a Numeric Pain Scale form. All 3 groups received the same treatment as they did in week 1 and nail temperature reached was taken with a surface thermometer.

At Stage 3 (Week 24), digital images of the toenails were taken to establish the severity and/or improvement of fungal infection. All patients received final standard podiatric treatment. Nail scrapings were then taken at the site of the eponychium for final PAS staining and fungal MC&S to identify whether fungal elements and the causative pathogen were still present at the site of new nail growth. Data was recorded on the last Patient Care Form.

Data and Analysis

Data recorded on the Patient Care Form and Numeric Pain Scale as well as the laboratory reports were grouped, sorted, and recorded on a Microsoft Excel® 2016 spreadsheet. All digital photography data was recorded on a Microsoft PowerPoint® 2016 presentation. Infection patterns were traced on the photos by using Microsoft Paint® 2016. Data were analysed using Sigmaplot, Version 13. Bar charts and line graphs were utilized to indicate the statistical significance between intra- and intergroup results. Statistical significance of all analysed data was recorded as $p \leq 0.05$ (*); $p \leq 0.01$ (**) and $p \leq 0.001$ (***) at Week 1, Week 12, and Week 24 for all groups. These hypotheses tests were used to test the validity of the results. A small p-value (<0.05) indicates strong evidence against the null hypotheses.

3. Results

Patient numbers included 23 (51.1%) female and 22 (48.9%) male, and the age ranged between 13 and 89 years, with a "mean of 59.60 (SD) 16.65". Overall, 19 (42.2%) patients had never sought treatment before and 26 (57.8%) had previous treatment done. These included oral Itraconazole, Griseofulvin, and Terbinafine Hydrochloride as well as topical Amorolfine, Fix-4-Nails and Tea Tree Oil. We classified the infections into the following groups: 1 (2.2%) PWSO; 7 (15.6%) WSO; 15 (33.3%) TDO; 20 (33.4%) DSO and 20 (44.4%) DLSO. 16 (35.6%) had a combination infection.

3.1. PAS Stain and Fungal MC&S

PAS stain yielded the following results: Week 1 (n = 45), 41 positive (91.1%), 4 negative (8.9%); Week 12 (n = 44), 9 positive (20%), 35 negative (80%) and Week 24 (n = 44), 1 positive (2.2%), 44 negative (97.8%). Fungal MC&S yielded the following results: Week 1, 20 positive (44.4%), 25 negative (55.6%); Week 12, 5 positive (11.1%), 39 negative (88.9%) and Week 24, 44 negative (100%). The authors were able to make a methodological consideration by

comparing the results obtained with the PAS staining, which allowed to better identify Onychomycosis (91.1%), with the microscopical approach (44.4%).

Different organisms identified at Week 1: 1 (2.2%) yeast infection (*Candida albicans*), 9 (20%) dermatophyte infections (*Trichophyton rubrum*) and 10 (22.2%) non-dermatophyte mould infections (*Fusarium* and *Curvularia species*), which is in contrast to literature, where most infections are dermatophyte in nature. Identifying organisms after one laser treatment (standard and new parameters) and control therapy at Week 12: 4 (9.09%) dermatophyte infections (*Trichophyton rubrum*) and one (2.2%) had a combination yeast and non-dermatophyte mould infection (*Fusarium species* and *Candida parapsilosis*). At Week 24, 1 (2.2%) patient had a positive PAS stain and no positive fungal MC&S results.

3.2. Overall Pain and Temperature Differences between Groups 2 and 3

For the laser treatment groups, patients were asked to record their pain during treatment between 1 (no pain) and 10 (excruciating pain). Temperatures reached directly after therapy were analysed and compared to pain experienced during treatment at Weeks 1 and 12 for both halluces (Figure 1) and lesser digits (Figure 2).

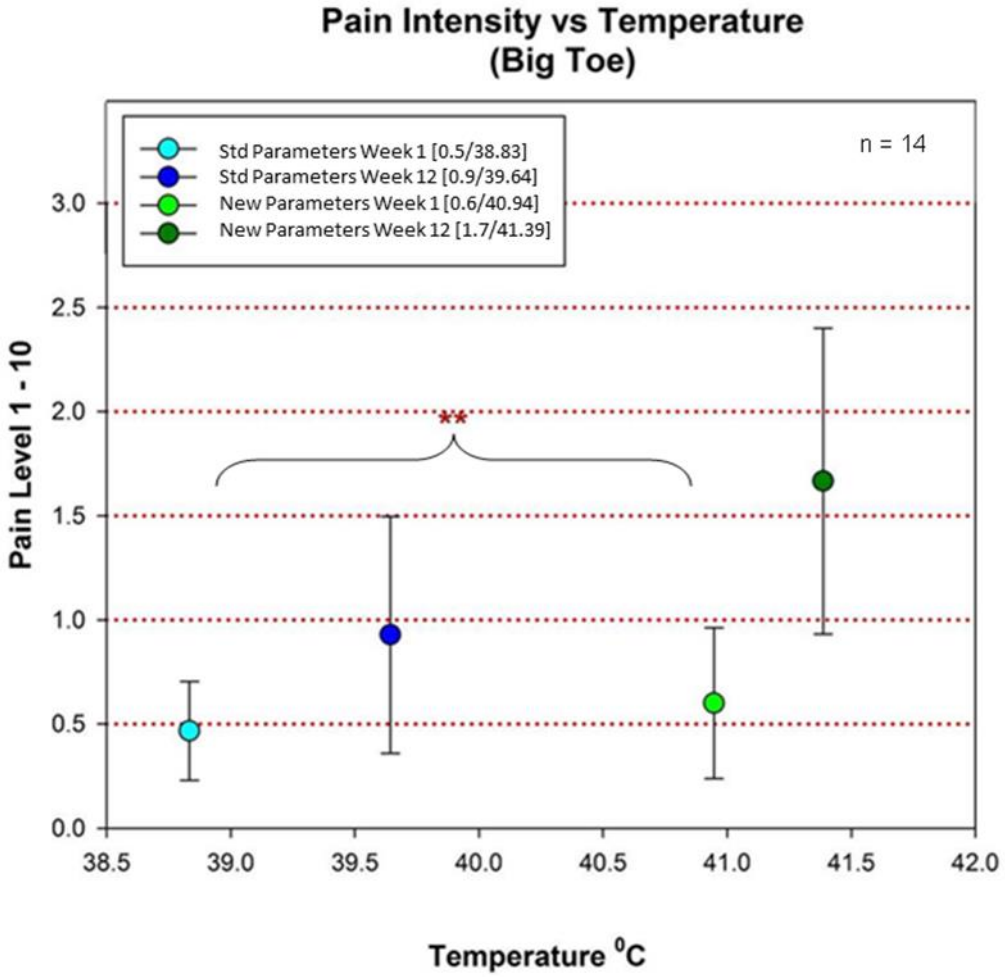

Figure 1. Comparing the halluces, both treatment groups were compared to each other, and each group between treatments (weeks). Statistical significances were indicated with a (*). Pain increases were indicated by black (*) and temperature increases were indicated by red (*). p-values of $p \leq 0.01$ (**) were noted between treatments and indicated that halluces temperature between Groups 2 and 3 was significantly increased at Week 1 and may be due to the increase in energy fluence for the new laser parameters set by us.

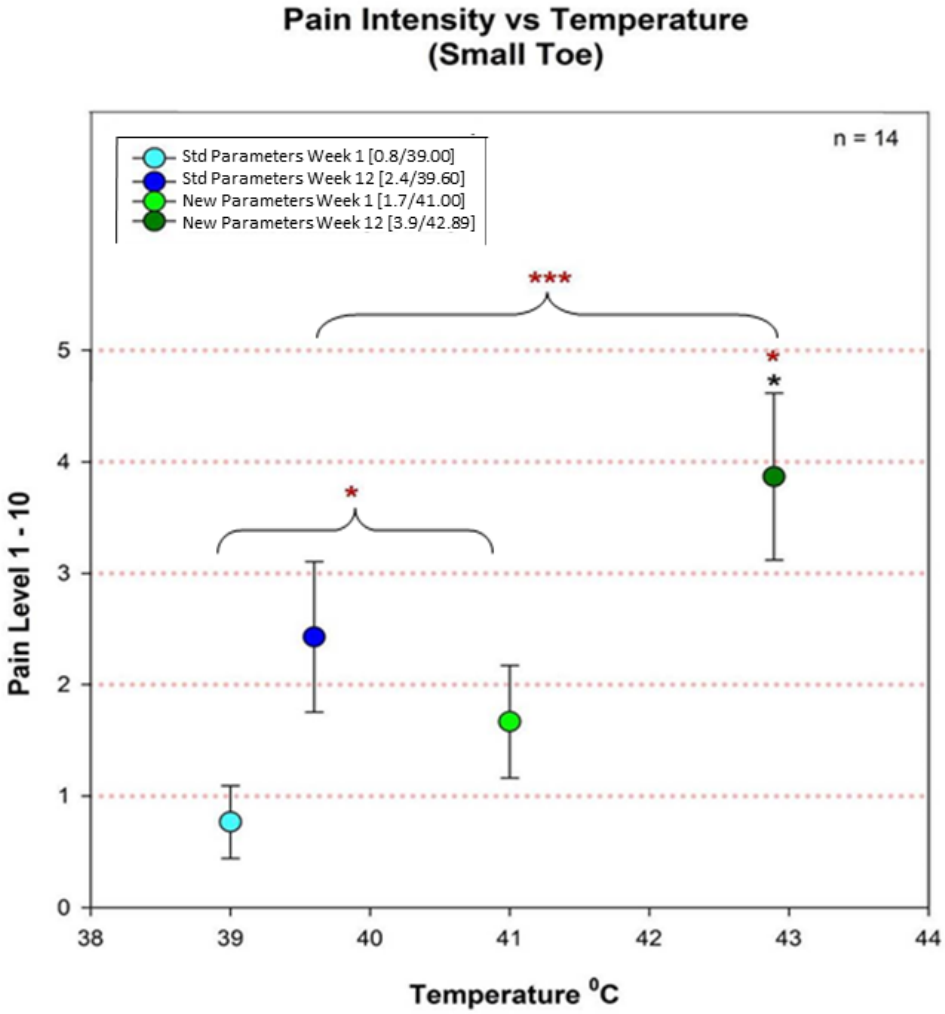

Figure 2. Comparing the lesser digits, both treatment groups were compared to each other, and each group between treatments (weeks). *p*-values of $p \leq 0.05$ (*) and $p \leq 0.001$ (***) were noted. In Group 3, there was a statistically significant increase ($p \leq 0.05$ (*)) in lesser digit temperature between Weeks 1 and 12 and was likely due to increased organism inactivity after one treatment. A significant increase ($p \leq 0.05$ (*)) in temperature was shown between Groups 2 and 3 at Week 1. As with the standard laser group, nail plate temperatures reached increased with the second laser treatment. The overall lesser digit pain experienced for this group showed a statistically significant result ($p \leq 0.05$ (*)) when compared to Week 1. When comparing Groups 2 and 3, results show a bigger statistically significant increase in lesser digit temperature ($p = 0.001$ (***)) at Week 12.

Although there were statistically significant increases in temperature and pain, these values were still relatively low. As such, all patients who received standard and new laser therapy reported no adverse effects during or after treatment. When asked whether the new laser parameters set by us was tolerable, all Group 3's patients indicated that it was.

3.3. Overall Treatment Results

Infections were classified according to the SCIO prior to treatment and scored between 1 (lowest) and 30 (highest). The SCIO only has recommendations for oral or topical treatment and with laser and light being investigated as a new source of safe and effective treatment, there may be an avenue for inclusion into the SCIO (Figure 3).

In Group 1, visually, there was an improvement of infection of 11 (73.3%) patients and 4 (26.7%) did not show improvement. Overall, results did not show a statistically

significant difference in SCIO and 26.7% of patients failed to respond to conventional (topical 5% Amorolfine) therapy for OM for the duration of this study (6 months).

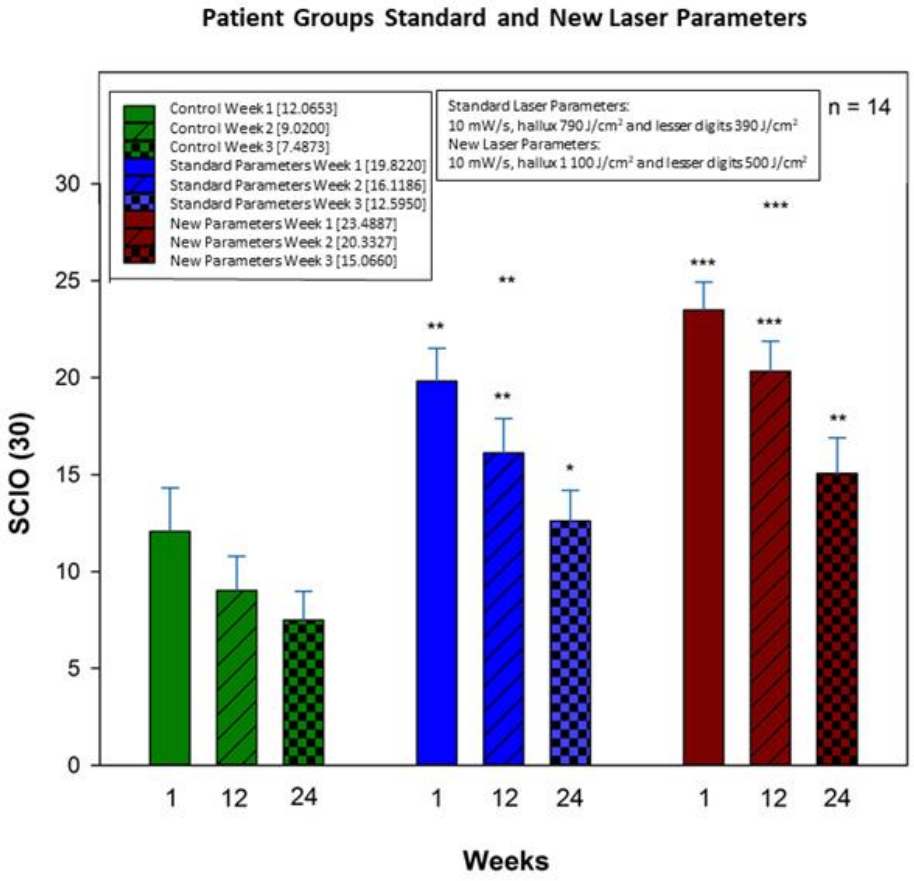

Figure 3. All treatment groups were compared to the control group (Group 1), to each other (Group 2 and 3), and each group between treatments (weeks). Statistical significances were indicated with a (*). p-values of $p \leq 0.05$ (*); $p \leq 0.01$ (**) and $p \leq 0.001$ (***) were noted between treatments. When comparing the need for treatment over 24 weeks, there was no significant change in Group 1, but there was a statistical significance $p \leq 0.01$ (**) for standard laser treatment and $p \leq 0.001$ (***) for new laser treatment. This indicates that the amount of treatment needed over time decreased in these groups.

In Group 2, there was an overall decrease in SCIO of 7.2270, which was statistically significant ($p \leq 0.01$ (**)) and may be due to the higher infection measurement at Week 1. Visually, there was an improvement of infection of 9 (64.3%) patients and 5 (35.7%) did not show improvement. Overall, results showed a statistically significant difference in infection measurement and 35.7% of patients failed to respond to standard laser therapy for OM for the duration of this study. This also indicates that standard laser therapy was not a better treatment option for OM infections when compared to conventional therapy.

Finally, in Group 3, there was an overall decrease in SCIO of 8.4227, which was the greatest statistically significant ($p \leq 0.001$ (***)) decrease. Visually, there was an improvement of infection of 13 (86.7%) patients and 2 (13.3%) did not show improvement. Overall, results showed the biggest statistically significant difference in infection measurement and 13.3% of patients failed to respond to new laser therapy for OM for the duration of the study. This also indicates that new laser therapy was the best treatment option for OM infections when compared to conventional and standard laser therapies.

OM infection cure rates are classified into clinical cure (visual), mycological cure (laboratory) and complete cure (visual and mycological). The overall results after 24 weeks

show an improvement in infection of 33 patients (75%). This improvement is based on the combined negative PAS stain results and the visual improvement after 6 months (Figure 4).

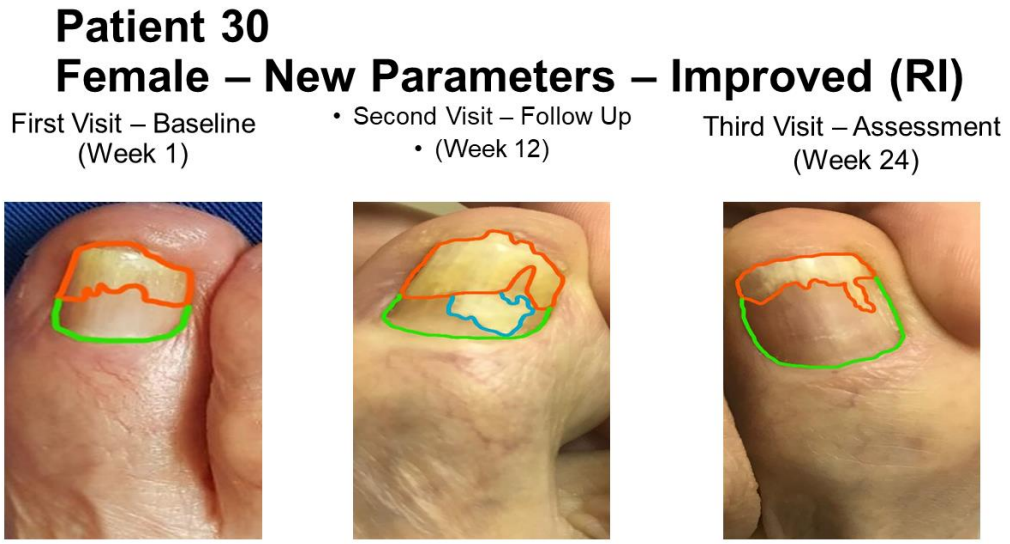

Figure 4. Objective image analysis of infected digits. The overall results indicate that the parameters set in this study was the most successful in treating OM infections. Example of a Group 3 patient (female, 85 years of age, infection duration 10 years, positive PAS stain) indicating overall improvement over 24 weeks.

Entering the results of the experiment, the data obtained would show that there was a statistical significance, $p \leq 0.01$ (**), for standard laser treatment compared with, $p \leq 0.001$ (***), new laser parameter treatment, drawing as an indication that the treatment needed over time decreased. No adverse effects were noted with new laser therapy. An 86.7% visual improvement was recorded in Group 3 after 24 weeks; In conclusion, the authors consider phototherapy as "the best treatment option for Onychomycosis" and consider establishing a "new protocol for the standardization of laser irradiation with the possible inclusion into the Scoring Clinical Index for Onychomycosis treatment plan".

4. Discussion

It is evident that OM is increasing in resistance against oral and topical antifungal agents. Laser therapy and light has been the topic of discussion for the treatment of OM; more specifically, the 1064 nm ND: YAG laser. Preliminary studies show good clinical and microscopical cure rates using the Nd: YAG 1064 nm laser in the short term. Published studies revealed poor, small study samples; no standard Podiatric treatment was done prior to laser treatment; not enough time given between treatments in order to see clear nail growth; limited output and no clear protocol could be attained during literature review. A clear protocol with three separate study groups were designed and proposed for the purpose of this study.

4.1. Standard Podiatric Treatment Efficacy

Standard podiatric treatment is important when deciding to treat OM, as the fungi denatures the nail keratin, causing increased nail thickness. This thickness decreases treatment outcomes as the product/therapy cannot penetrate the desired structures. A study conducted by Malay looked at the different outcomes in 55 patients between nail debridement alone and nail debridement with a topical antifungal nail lacquer. The primary outcome was mycological cure. Of the 55 patients, 27 (49.09%) were allocated to the debridement only group, and 28 (50.91%) were allocated to the debridement and antifungal group. At 10 months and 2 weeks follow-up, 76.74% of patients achieved

mycological cure in the antifungal group. None experienced mycological cure in the control group [35]. Recently, Fernández et, al. did a study where they combined laser and ozone therapy for OM in vitro and *ex vivo*. In the ex vivo model experiments, with the same duration and ozone concentration, *Penicillium chrysogenum* and *Epidermophyton floccosum* showed total inhibition; *Trichophyton mentagrophytes* and *Trichophyton rubrum* showed a 75% growth inhibition; *Microsporum canis* showed a delay in sporulation; and *Microascus brevicaulis* and *Aspergillus terreus* did not show growth inhibition. This combined laser and ozone treatment may be developed as a fast therapy for human onychomycosis, as a potential alternative to the use of antifungal drugs with potential side effects and long duration treatments [36]. This supports our reasoning for doing standard podiatric treatment for all patients and likely contributed to the results, as the nails were etched thin enough for treatment to penetrate.

4.2. Specimen Sampling and Analysis

Reported studies support the findings in our study where PAS stain identified OM infections in 91.1% of samples, compared to 44.4% in culture form [7,8,13,14,35]. This was a culture failure rate of 55.6%. Although PAS staining shows good sensitivity rates, the specificity of this test is low. This is a clinical concern when prescribing an oral or topical antifungal agent, as these drugs act against certain fungal elements. Culture results need to be present when prescribing a specific antifungal agent. However, laser therapy works on the principle of denaturing a fungal elements membrane potential through heat. When a practitioner is considering laser therapy as a treatment option, culture results are not necessary. It is recommended to do both PAS staining (as primary test) and Fungal Microscopy (as secondary test) when doing combination therapy.

4.3. Infective Organisms

In earlier published studies, OM was primarily caused by dermatophytes (60–90%), followed by yeasts and rarely by non-dermatophyte moulds [5,7,8,11–13], which is inconsistent with our study where the primary infective agent was non-dermatophyte moulds (22.2%), followed by dermatophyte infections (20%) and yeasts (2.2%). As reported by Kaur, et al., these previously regarded contaminants are now increasingly recognized as primary causative pathogens [10]. However, careful diagnostic attention is required when identifying non-dermatophyte moulds as aetiologic agents. The biggest difference between a non-dermatophyte mould infection and others, is that the mould should be the sole (or primary) aetiologic agent in culture and can only be achieved by repeated reproducibility growths at different time points and that dermatophytes do not appear on repeated culture attempts [37].

4.4. SCIO Decrease

In Group 1, there was an overall infection measurement decrease of 4.578, which correspond with literature [28,38]. Group 2 showed an overall infection measurement decrease of 7.2270, which was statistically significant ($p \leq 0.01$ (**)) and may be due to the higher SCIO at Week 1. Finally, in Group 3, there was an overall infection measurement decrease of 8.4227, and it was the biggest statistically significant ($p \leq 0.001$ (***)) decrease compared to standard laser and control groups. This may prove an accurate indicator of therapeutic effectiveness, but further clinical studies must be done before definitive claims can be made [30]. As we aimed to identify the effectiveness of laser therapy for OM compared to topical treatment, this may bridge the gap of clinical effectiveness by adding laser therapy as a possible route of treatment, depending on the SCIO. Ethical clearance was granted. Helou et, al. concluded in their study of 105 patients, where they looked at the response of big toenail OM to 1064 nm Nd: YAG laser treatment that the SCIO decrease after 3 sessions of Nd: YAG laser was significantly more important in women and in patients with positive mycology culture, smaller affected area of the nail, no subungual hyperkeratosis, and no nail matrix involvement. Age, smoking, hypertension, and side

effects were not shown to significantly correlate with the decrease of the SCIO score [39], which is similar to the findings of Kandpal et, al. [40]. Both of the findings in these studies correlate with the findings in our study where there was a significant decrease in the SCIO with the proposed new laser parameter settings.

4.5. Temperature and Pain Correlation

When looking at laser therapy as a potential alternative treatment for OM, the probable pain associated with it has to be taken into consideration. The type of laser chosen to make the comparison is justifiable for their intrinsic characteristics and for the literature data that report the use of 1064 nm ND: YAG laser. "Preliminary studies show good clinical and microscopical cure rates using the Nd: YAG 1064 nm laser in the short term" [2,4,12,18,20]. In Group 2, the mean hallux temperature reached was 38.83 degrees Celsius at Week 1 and 39.64 at Week 12, and the mean hallux pain experienced at Week 1 was 0.5/10 and 0.9/10 for Week 12. Both increases were not significant for standard laser therapy. In Group 3, the mean hallux temperature reached was 40.94 degrees Celsius at Week 1 and 41.39 at Week 12, and the mean hallux pain experienced at Week 1 was 0.6/10 and 1.7/10 for Week 12. Also, both increases were not significant for new laser therapy. When comparing Week 1 temperatures between Groups 2 and 3, there was a statistically significant increase ($p \leq 0.01$ (**)) and may be due to the increase in energy fluence for the new laser parameters set by us as well as the inactivity of fungal elements after the initial treatment. As for the lesser digits in Group 2, the mean toe temperature reached was 41.00 degrees Celsius at Week 1 and 42.89 at Week 12 and was a statistically significant increase ($p \leq 0.05$ (*)). There was also a significant increase ($p \leq 0.05$ (*)) in temperature between Groups 2 and 3 at Week 1. When comparing Groups 2 and 3, there was a bigger statistically significant increase in temperature ($p = 0.001$ (***)) at Week 12. The mean lesser digit pain experienced at Week 1 was 1.7/10 and 3.9/10 for Week 12, which was also statistically significant ($p \leq 0.05$ (*)) when compared to Week 1 in Group 3. Our findings are supported by a study conducted by Kozarev, where 72 patients were treated with a long-pulse 1064 nm Nd: YAG laser, with a fluence of 35–40 J/cm^2; 4 mm spot size; pulse duration 35 ms. During treatment, the nails reached 50 degrees Celsius and was at 40 degrees Celsius after one minute. Patient pain was reported as follows: 24 (33.33%) no pain; 35 (48.61%) mild pain; 13 (18.06%) moderate pain; 0% severe pain; 0% intolerable. Kozarev also reported that the desired average tissue temperature for laser irradiation is between 43–50 degrees Celsius, which support the findings in our study [41]. Tolerating higher temperatures is in correlation with increased blood flow and may explain why patients experienced less pain tolerance at the second treatment. Heating the fungal colonies does not instantly kill them, but it results in the disability of them to replicate or survive according to an apoptotic mechanism. Killing of the fungal colonies may be caused by superheating and exploding or rupturing the fungal cell membranes [41].

4.6. Overall Findings

In the control group, 11 (73.3%) patients showed improvement and 4 (26.7%) failed to respond and this could be due to patient non-adherence to protocol given regarding adequate decontamination of shoes, socks, etc. at home, or failure to apply the Amorolfine once weekly. In another study conducted by Gupta, et al., they did an evaluation of 26 studies and found that 5% topical Amorolfine was effective in mild to moderate OM, and they also had to apply the treatment to the affected nails once weekly. Here, two open, randomized studies compared the efficacy and safety between once-weekly applications and twice-weekly applications. Both studies found slightly higher cure rates in the twice-weekly groups; however, there was no statistical significance between the dosage regimens [42]. Another study conducted by Ghannoum and Isham stated that low efficacy rates can mainly be attributed to the inability of the drug to penetrate the nail plate and bed where the infection resides [19]. This study's 5% topical Amorolfine outcomes are better,

when compared to another study by Roberts, et al., which indicate only a 50% efficacy for fingernail and toenail OM [20].

In the standard laser therapy group, 9 (64.3%) patients showed improvement and 5 (35.7%) failed to respond. Interestingly, this indicates that standard laser therapy was not a better treatment option for OM infections compared to conventional therapy, although the SCIO decreased more between standard laser therapy patients compared to control patients. However, this could be due to patient non-adherence to decontamination protocol given for shoes, socks, etc. This was the original research problem—that standard (industry set) laser parameters were not effective in treating OM. Our findings are supported by Hollmig, et al., where the primary end point was a negative mycological cure from all clinically involved nails. Laser parameters (recommended by manufacturer, as was the case with standard laser settings in our study) included: 1064 nm; fluence of 5 J/cm^2; pulse width 0,3 ms; spot size 6 mm and rate of 6 Hz to achieve measured target temperatures of between 40–42 degrees Celsius. 12 laser patients completed the study at 3 months, and only 50% had negative fungal cultures. At 12 months follow up, no modest improvement of nail plate clearance was sustained [43–46].

In the final group, who had new laser parameters set by us, 13 (86.7%) patients showed improvement and 2 (13.3%) failed to respond. These results also indicate that new laser therapy was the best treatment option for OM infections compared to conventional and standard laser therapies. The overall SCIO for this group decreased the most. Our results were more effective when compared to a study conducted by Zhang, et al., who used long-pulse 1064 nm laser therapy on 33 patients with clinically and mycologically proven OM. All patients were given 8 sessions at 1-week intervals. At week 8, 68% of nails showed mycological cure [43]. Our study results are also higher than those of Wantiphakdeedecha, et al., where 35 patients demonstrated an overall mycological cure rate of 51.9% at 6 months [31,44,47,48].

The results at week 24 show an overall improvement in infection of 33 patients (75%). This improvement is based on the combined negative PAS stain results and the visual improvement after 6 months. There was no difference in overall improvement regardless of treatment between week 12 and 24; however, there was a difference between individual group improvement rates. All patients who received standard and new laser therapy reported no adverse effects during or after treatment. When asked whether the laser parameters set for this study was tolerable, both group's patients indicated that it was. Summarizing, during the treatments of the three groups which lasted for 12 weeks, with the use of lasers, a temperature higher than 50 °C was never reached, nor a pain index higher than mild pain. The authors, based on the results and literature data, propose as the antifungal mechanism of the laser treatment used the fact that the "Heating the fungal colonies does not instantly kill them, but it results in the disability of them to replicate or survive according to an apoptotic mechanism. The killing of the fungal colonies may be caused by superheating and exploding or rupturing the fungal cell membranes [33]. From the findings, a new protocol for the standardization of laser therapy with the possible inclusion into the SCIO treatment plan, has been proposed.

5. Conclusions

In conclusion, the authors state that onychomycosis, due to the new pharmacological resistance and the risk of hepatotoxicity of oral therapies, is a current problem from a therapeutic point of view. Thus, new non-pharmacological or non-pharmacological therapeutic approaches must be taken into consideration. We aimed to set new laser parameters that were effective in treating OM and we also wanted to ascertain whether these parameters were tolerable and safe for patients. The proposed new laser parameters were subjected to clinical verification and the data obtained gave indications of a better therapeutic activity both of the parameters previously used with the same type of laser, and of the standard topical antifungal therapy. The findings indicate that new parameters were more effective at 6 months, compared to other methods topical antifungal therapy

and standard laser therapy. A new protocol for the standardization of laser therapy has been proposed with the possible inclusion into the SCIO treatment plan.

Author Contributions: Conceptualization, N.D. and H.A.; methodology, formal analysis, N.D.; writing—original draft preparation, N.D.; writing—review and editing, N.D. and H.A.; supervision, H.A.; project administration, H.A.; funding acquisition, H.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work is based on the research supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant No 98337). The authors sincerely thank the University of Johannesburg, the National Laser Centre, and the National Research Foundation of South Africa for their financial grant support.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the University of Johannesburg Research Ethics Committee (Approval number: REC-01-176-2015 Date: 3 June 2015).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy issues.

Acknowledgments: The authors would like to acknowledge the Institution, University of Johannesburg, for the opportunity to conduct this study under its auspices.

Conflicts of Interest: The authors declare no conflict of interest.

Ethical Approval: Ethical approval has been granted for studies involving human subjects by the University of Johannesburg Faculty of Health Sciences Faculty Ethics Committee on 3 June 2015, reference number REC-01-176-2015. Furthermore, all patients who participated in this study gave formal consent for the outcomes of this study to be published, with images.

Abbreviations

OM	Onychomycosis
DSO	Distal Subungual Onychomycosis
DLSO	Distolateral Subungual Onychomycosis
PSO	Proximal Subungual Onychomycosis
PWSO	Proximal White Superficial Onychomycosis
TDO	Total Dystrophic Onychomycosis
WSO	White Superficial Onychomycosis
SCIO	Scoring Clinical Index for Onychomycosis
Nd: YAG	Neodymium Yttrium Aluminium Garnet
FDA	Food and Drug Administration
KOH	Potassium Hydroxide
PAS	Periodic Acid-Schiff
MC&S	Fungal microscopy and culture

References

1. Baran, R.; Kaoukhov, A. Topical antifungal drugs for the treatment of onychomycosis: An overview of current strategies for monotherapy and combination therapy. *J. Eur. Acad. Dermatol. Venereol.* **2005**, *19*, 21–29. [CrossRef]
2. Kimura, U.; Takeuchi, K.; Kinoshita, A.; Takamor, K.; Hirum, M.; Suga, Y. Treating onychomycosis of the toenail: Clinical efficacy of the sub-millisecond 1064 nm Nd. YAG laser using a 5 mm spot diameter. *J. Drugs Dermatol.* **2012**, *11*, 496–504. [PubMed]
3. Ortiz, A.E.; Avram, M.M.; Wanner, M.A. A review of lasers and light for the treatment of onychomycosis. *Lasers Surg. Med.* **2014**, *46*, 117–124. [CrossRef]
4. Suga, Y.; Kimura, U.; Hiruma, M. Can persistent toenail fungus be successfully treated with laser? *J. Med. Mycol.* **2014**, *55*, J65–J71. [CrossRef] [PubMed]
5. Tcherney, G.; Penev, P.K.; Nenoff, P.; Zisova, L.G.; Cardoso, G.C.; Taneva, T.; Ginter-Hanselmayer, G.; Ananiev, J.; Gulubova, M.; Hristova, R.; et al. Onychomycosis: Modern diagnostic and treatment approaches. *Wien. Med. Wochenschr.* **2013**, *163*, 1–12. [CrossRef] [PubMed]

6. Tosti, A. Efinaconazole solution 10%: Topical antifungal therapy for toenail onychomycosis. *Cutis* **2013**, *92*, 203–208. [PubMed]
7. Welsh, O.; Vera-Cabrera, L.; Welsh, E. Onychomycosis. *Clin. Dermatol.* **2010**, *28*, 151–159. [CrossRef]
8. Nenoff, P.; Grunewald, S.; Paasch, U. Laser therapy of onychomycosis. *J. Der Dtsch. Dermatol. Ges.* **2014**, *12*, 33–38. [CrossRef]
9. Waibel, J.; Wulkan, A.J.; Rudnick, A. Prospective efficacy and safety evaluation of laser treatments with real-time temperature feedback for fungal onychomycosis. *J. Drugs Dermatol.* **2013**, *12*, 1237–1242.
10. Kaur, R.; Kashyap, B.; Bhalla, P. Onychomycosis—Epidemiology, diagnosis and management. *Indian J. Med. Microbiol.* **2008**, *26*, 108–116. [CrossRef]
11. Evans, E.G. Causative pathogens in onychomycosis and the possibility of treatment resistance: A review. *J. Am. Acad. Dermatol.* **1988**, *38*, S32–S36. [CrossRef]
12. Gupta, A.K.; Paquet, M.; Simpson, F.C. Therapies for the treatment of onychomycosis. *Clin. Dermatol.* **2013**, *31*, 544–554. [CrossRef] [PubMed]
13. Noguchi, H.; Miyata, K.; Sugita, T.; Hiruma, M.; Hiruma, M. Treatment of onychomycosis using a 1064 nm Nd. YAG laser. *J. Med. Mycol.* **2013**, *54*, 333–339. [CrossRef] [PubMed]
14. Nenoff, P.; Krűger, C.; Ginter-Hanselmayer, G.; Tietz, H.J. Mycology—An update. Part 1: Dermatomycosis: Causative agents, epidemiology and pathogenesis. *J. Ger. Soc. Dermatol.* **2014**, *1203*, 188–210. [CrossRef]
15. Nenoff, P.; Krűger, C.; Ginter-Hanselmayer, G.; Schulte-Beerbühl, R.; Tietz, H.-J. Mycology—An update. Part 2: Dermatomycosis: Clinical picture and diagnosis. *J. Ger. Soc. Dermatol.* **2014**, *1209*, 749–777.
16. Thomas, J.; Jacobson, G.A.; Narkowicz, C.K.; Peterson, G.; Burnet, H.; Sharpe, C. Toenail onychomycosis: An important global disease burden. *J. Clin. Pharm. Ther.* **2010**, *35*, 497–519. [CrossRef]
17. Baudraz-Rosselet, F.; Ruffieux, C.; Lurati, M.; Bontems, O.; Monod, M. Onychomycosis insensitive to systemic terbinafine and azole treatments reveals non-dermatophyte molds as infectious agents. *Dermatology* **2010**, *220*, 164–168. [CrossRef]
18. Westerberg, D.P.; Voyack, M.J. Onychomycosis: Current trends in diagnosis and treatment. *Indian J. Clin. Pract.* **2014**, *25*, 309–319.
19. Ghannoum, M.; Isham, N. Fungal nail infections (onychomycosis): A never-ending story? *PLoS Pathog.* **2014**, *10*, 1–5. [CrossRef]
20. Roberts, D.T.; Taylor, W.D.; Boyle, J. Guidelines for treatment of onychomycosis. *Br. J. Dermatol.* **2003**, *148*, 403–410. [CrossRef]
21. Sergeev, A.Y.; Gupta, A.K.; Sergeev, Y.V. The scoring clinical index for onychomycosis (SCIO Index). *Ski. Ther. Lett.* **2002**, *7*, 6–7.
22. Gupta, A.K.; Simpson, F.C. New therapeutic options for onychomycosis. *Expert Opin. Pharmacother.* **2012**, *13*, 1131–1142. [CrossRef] [PubMed]
23. Wilsmann-Theis, D.; Sareika, F.; Bieber, T.; Schmid-Wendtner, M.-H.; Wenzel, J. New reasons for histopathological nail clipping examination in the diagnosis of onychomycosis. *J. Eur. Acad. Dermatol. Venereol.* **2011**, *25*, 235–237. [CrossRef]
24. Zaias, N. Onychomycosis. *Arch. Dermatol.* **1972**, *105*, 263–274. [CrossRef]
25. Bristow, I.R. The effectiveness of lasers in the treatment of onychomycosis: A systemic review. *Br. J. Foot Ankle Res.* **2014**, *7*, 1–10. [CrossRef] [PubMed]
26. Evans, E.G. Resistance of Candida species to antifungal agents used in the treatment of onychomycosis: A review of current problems. *Br. J. Dermatol.* **1999**, *141*, 33–35. [CrossRef]
27. Bunert, N.; Homey, B.; Gerber, P.A. Onychomycosis. Successful treatment with a 1064-nm Nd. YAG laser. *Der Hautarzt* **2013**, *64*, 716–718. [CrossRef]
28. Gupta, A.K.; Paquet, M. A retrospective chart review of the clinical efficacy of Nd. YAG 1064-nm laser for toenail onychomycosis. *J. Dermatol. Treat.* **2014**, *5*, 1–3.
29. Heers, H.; Jäger, M.W.; Raulin, C. Treatment of onychomycosis using the 1064 nm Nd. YAG laser: A clinical pilot study. *J. Der Dtsch. Dermatol. Ges.* **2014**, *12*, 322–329.
30. Wantiphakdeedecha, R.; Thanomkitti, K.; Bunyaratavej, S.; Manuskiatti, W. Efficacy and safety of 1064 nm Nd: YAG laser in the treatment of onychomycosis. *J. Dermatol. Treat.* **2015**, *27*, 75–79. [CrossRef]
31. Dembskey, N.; Abrahamse, H. Laser Therapy for the Treatment of Onychomycosis: Best Evidence Based Practice or Not? *Clin. Res. Foot Ankle* **2016**, *4*, 3–7. [CrossRef]
32. Malay, D.S. Efficacy of debridement alone versus debridement combined with topical antifungal nail lacquer for the treatment of pedal onychomycosis: A randomised, controlled trial. *J. Foot Ankle Surg.* **2009**, *48*, 294–308. [CrossRef]
33. Gupta, A.K.; Ryder, J.E.; Summerbell, R.C. The diagnosis of non-dermatophyte mold onychomycosis. *Int. J. Dermatol.* **2003**, *24*, 272–273. [CrossRef] [PubMed]
34. Sigurgeirsson, B.; Olafsson, J.H.; Steinsson, J.T.; Kerrouche, N.; Sidou, F. Efficacy of amorolfine nail lacquer for the prophylaxis of onychomycosis over 3 years. *J. Eur. Acad. Dermatol. Venereol.* **2010**, *24*, 910–915. [CrossRef] [PubMed]
35. Aditya, K. Gupta, Maanasa Venkataraman, Emma M Quinlan. Efficacy of lasers for the management of dermatophyte toenail onychomycosis. *J. Am. Podiatr. Med. Assoc.* **2021**, 20–236. [CrossRef]
36. Ameen, M.; Lear, T.J.; Madan, V.; Mustapa, M.F.M.; Richardson, M. British Association of Dermatologists' guidelines for the management of onychomycosis 2014. *Br. J. Dermatol.* **2014**, *171*, 937–958. [CrossRef]
37. Gupta, A.K.; Jain, H.C.; Lynde, C.W.; Macdonald, P.; Cooper, E.A.; Summerbell, R.C. Prevalence and epidemiology of onychomycosis in patients visiting physicians' offices: A multicenter Canadian survey of 15,000 patients. *J. Am. Acad. Dermatol.* **2000**, *43* (Pt 1), 244–248. [CrossRef]
38. Fernández, J.; Del Valle Fernández, I.; Villar, C.J.; Lombó, F. Combined laser and ozone therapy for onychomycosis in an in vitro and ex vivo model. *PLoS ONE* **2021**, *16*, e0253979. [CrossRef]

39. Kandpal, R.; Arora, S.; Arora, D. Study of Q-switched Nd: YAG Laser versus Itraconazole in Management of Onychomycosis. *J. Cutan. Aesthet. Surg.* **2021**, *14*, 93–100.
40. Kosarev, J. Novel laser therapy in the treatment of onychomycosis. *J. Laser Health Acad.* **2010**, *1*, 1–8.
41. Gupta, A.K.; Simpson, F.C. Device-based therapies for onychomycosis treatment. *Skin Ther. Lett.* **2012**, *17*, 57–61.
42. Hollmig, T.; Rahman, Z.; Henderson, M.T.; Rotatori, R.M.; Gladstone, H.; Tang, J.Y. Lack of efficacy with 1064 nm Nd: YAG laser for the treatment of onychomycosis: A randomised, controlled trial. *J. Am. Acad. Derm.* **2014**, *70*, 911–917. [CrossRef] [PubMed]
43. Zhang, R.N.; Wang, D.K.; Zhuo, F.L.; Duan, X.-H.; Zhang, X.-Y.; Zhao, J.-Y. Long-pulse Nd: YAG 1064 nm laser treatment of onychomycosis. *Chin. Med. J.* **2012**, *125*, 3288–3291. [PubMed]
44. Hochman, L.G. Laser treatment of onychomycosis using a novel 0.65-milisecond pulsed Nd:YAG 1064 nm laser. *J. Cosmet. Laser Ther.* **2011**, *13*, 2–5. [CrossRef] [PubMed]
45. Sotiriou, E.; Koussidou-Ermonti, T.; Chaidemenos, G.; Apalla, Z.; Ioannides, D. Photodynamic therapy for distal and lateral subungual toenail onychomycosis caused by Trichophyton rubrum: Preliminary results of a single-centre open trial. *Acta Derm. Venerol.* **2010**, *90*, 216–217. [CrossRef]
46. Shemer, A.; Davidovici, B.; Grunwald, M.H.; Lyakhovitsky, A.; Amichai, B. Onychomycosis: A simpler in-office technique for sampling specimens. *J. Fam. Pract.* **2012**, *61*, 552–554.
47. Gupta, A.K.; Ryder, J.E.; Baran, R. The use of topical therapeutics to treat onychomycosis. *Derm. Clin.* **2003**, *21*, 481–489. [CrossRef]
48. Helou, J.; Maatouk, I.; Soutou, B. Big toenail onychomycosis features associated with response to 1064 nm Nd: YAG laser treatment. *J. Cosmet. Derm.* **2021**. [CrossRef]