Nd:YAG LASER TREATMENT OFONYCHOMYCOSIS

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Introduction: Onychomycosis

Onychomycosis is a fungal infection of the toenails and fingernails that results in thickening, discoloration, splitting of the nails, as well as lifting of the nails from the nail bed.
NAIL FUNGUS
(ONYCHOMYCOSIS)

- It has increased by 18.5% during the past several decades
- One-third of people with diabetes develop nail fungus
- Antibiotics & corticosteroids increase risk of fungal infections
- Artificial nails and overzealous manicuring contribute to nail fungus development
- 48% of the population may be affected by age 70
A characteristic feature of dermatophyte infections is an inflammatory pattern at the edge of the skin lesion, noted by redness and scaling or occasionally, blister formation.
Treatment options

- Multiple therapies, topical and oral methods, including surgical treatments, chemical ablation, and new laser treatments.
- Oral therapies result in better outcomes.
- However, none of these treatment options provides lasting high cure rates.
- Laser treatments are new modalities including waveleth of 1064 nm, 930 nm and 870 nm, PDT, femtosecond 800nm laser...
## Laser systems, wavelengths, and fluences used during the initial phase of the study

<table>
<thead>
<tr>
<th>Laser system</th>
<th>Wavelength (nm)</th>
<th>Fluence (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intense pulsed light (Lumenis)</td>
<td>695 to 1,000</td>
<td>38, 45, 57</td>
</tr>
<tr>
<td>Intense pulsed light (Lumenis, )</td>
<td>755 to 1,000</td>
<td>38, 45, 57</td>
</tr>
<tr>
<td>Pulsed dye laser (Candela)</td>
<td>585</td>
<td>8, 11, 14</td>
</tr>
<tr>
<td>Q-switched Nd:YAG laser (Surgical Laser Technology)</td>
<td>532</td>
<td>8, 10</td>
</tr>
<tr>
<td>Q-switched Nd:YAG laser (Surgical Laser Technology)</td>
<td>1,064</td>
<td>6, 8, 10, 12</td>
</tr>
<tr>
<td>Erbium YAG laser (Sciton)</td>
<td>2,940</td>
<td>25</td>
</tr>
<tr>
<td>KTP laser (Laserscope)</td>
<td>532</td>
<td>2, 4, 6, 8</td>
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Treatment with 1064nm laser at 4 and 8 J/cm² significantly reduced fungal growth rate compared to the average growth of all laser treated colonies.
Aim of Study:

To investigate (in vitro and in vivo) topical near infrared (1064 nm wavelength) photoinactivation of T. Rubrum, T. Mentagrophytes, Aspergilus niger, Candida sp., and molds as a major fungi that cause onychomycosis and superficial fungal skin infection.
Study Design: Inclusion study criteria:

Toenail fungus, finger nail fungal infection in all four clinical types of fungal nail infection
- total dystrophic form,
- distal subungual onychomycosis,
- proximal subungual onychomycosis,
- endonyx onychomycosis

Superficial skin fungal infection (tinea cutis glabrae) with nail involvement.
Ages between 18-45 years old.
Signed informed consent before laser procedure.
Exclusion study criteria:

- Treatment of systemic antifungal therapy or oral antifungals 6 months before laser procedure.
- Treatment with local antifungals.
- Usage of local antifungal therapy such as Castellani sol., which are changing nails pigmentation.
- Usage of nail coloring dyes or photosensibilisators which are changing nails pigmentation.
- Subungual exostosis.
Exclusion study criteria: cont.

- Pregnancy
- Existence of subungual hematoma or nevoid subungual formation, bacterial nail infection which are changing nail pigmentation.
- Existence of concomitant nail disorders such as psoriasis of nail plate, lichen planus and atopic dermatitis.
Special attention was made about factor which cause additional nail pigmentation

- Nail polishes which may contain magnesium or iron
- Professional exposure to dyes and asphalt
Attention was made about other drugs:

- **Vasodilators** which are increasing the blood flow through the nail region (and thus enable the quicker cooling)

- **Warfarin therapy** according to INR data
Special attention was made about factor which cause or increase skin photosensitivity and additional changes in skin pigmentation.

- Systemic therapy with photosensitive effect:
  Retinoids, cephalosporins, griseofulvin, naldiksic acid, tetraciklinls, hinolons, NSAIM, antimalarics, antimitotics, diuretics (furosemid, triamteren), trankvilisers, bensodiasepins, antiaritmics,sulfonamids...

- Local chemical photosensitisers: Arsen, busulfan, mechloretamine, 5 fluorouracil...
Methode:
Fungal nail infection

- 72 patients, 194 affected nails
- 162 patients, 413 infected nails

Follow up was performed at 3, 6 and 12 months, with mycological check ups at 3 and 6 months.
Fungal superficial skin infection

12 patients with tinea

36 patients with tinea

<table>
<thead>
<tr>
<th>Number of affected patients</th>
<th>Localisation of tinea</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

Follow up was performed at 2, 4, 8 and 12 weeks.
Methode:

- Sample collection process performed thorough cleansing of the nail area with alcohol to remove contaminants.
- The abnormal nail was clipped proximally and the nail bed and underside of the nail plate were scraped with a 1-2 mm serrated curette.
- The sampled material was divided into two portions: one for direct microscopy and the remainder for culture.
Direct microscopy: 10% KOH with 40% DMSO solution was used for direct microscopic examination

Candida sp.

Trichophyton rubrum

Trichophyton mentagrophytes

Molds
Diagnostic Tests:

Fungal Cultures

- DTM (Dermatophyte Test Medium)
  - Yellow to red is (+).
- Nickerson’s Media
  - Yeast
  - Black growth is (+)
- Sabouraud’s Media
  - Molds
Pre laser treatment nail preparation

Reduction of hyperkeratosis

- Er:YAG ablation
- Chemical keratin melting (urea)
- Dermabrader or sanpaper nail plate abrasion
**LP Nd:YAG laser settings**

- Long pulse 1064 nm wavelength laser (Fotona Dualis SPII platform)
- Fluence in the range of about 35 to 40 J/cm²
- Spot size of 4 mm
- Pulse width 25-35 ms
- Pulse rate 1 Hz

The fluence can be selected based on the thickness of the nail to be treated, a thicker nail can require a higher fluence.

Laser beam is applied to substantially the entire nail plate.
Application of laser beam

- Laser beam is applied by moving the beam in spiral pattern.
- After substantially all of the entire nail plate was irradiated, the treatment is stopped for a minute and repeated by going over the nail two more additional times.
- Each treatment consisted of moving the laser beam over the nail for three cycles, once per week, for 4 total treatments. (2)
- No topical medicine was applied to the nail.
### Results:
Clinical types of fungal nail infection in treated group

<table>
<thead>
<tr>
<th>Type of onychomycosis</th>
<th>Number of patients (%)</th>
<th>2009.</th>
<th>2011.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dystrophic</td>
<td>6 (8.3%)</td>
<td>13 (8.02%)</td>
<td></td>
</tr>
<tr>
<td>Distal subungual</td>
<td>38 (52.8%)</td>
<td>86 (53%)</td>
<td></td>
</tr>
<tr>
<td>Proximal subungual</td>
<td>22 (30.5%)</td>
<td>34 (21%)</td>
<td></td>
</tr>
<tr>
<td>Endonyx</td>
<td>6 (8.3%)</td>
<td>29 (17.9%)</td>
<td></td>
</tr>
</tbody>
</table>
Resultes of fungal nail isolates in a primary isolation medium Sabouraud Peptone-Glucose Agar

<table>
<thead>
<tr>
<th>Type of fungal isolates</th>
<th>Number of patients 2009.</th>
<th>Number of patients 2011.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida sp.</td>
<td>10 (13.9%)</td>
<td>94 (58%)</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>37 (51.4%)</td>
<td>40 (24.7%)</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>22 (30.5%)</td>
<td>24 (14.8%)</td>
</tr>
<tr>
<td>Aspergilus niger</td>
<td>3 (4.16%)</td>
<td>4 (2.5%)</td>
</tr>
</tbody>
</table>
Petri dish with T. mentagrophytes before and 3 days after LP NdYAG laser irradiation.

Note the inhibition of growth in treated colonies.
At high radiant energy each pulse creates an obvious crater.

At lower radiant energies blanching appears in the center of the beam on the target surface and a small plume of ablated material is emitted from the target surface.
Treatment efficacy (according to microscopy findings) at four control points: 3, 6, 9 and 12 months (2011, No of patients: 162)
Duration of follow-up intervals of interviewed patients (2011, No of patients: 162)
Subjective evaluation of results

- All interviewed patients reported to have no problems with nails after the treatment. 93,5% of them reported to have fully clear nail plates, while 6,5% of responders were not sure if their nail plates are fully clear.
Our idea from the start of the study:
Single shot generated aprox. 45-50°C, after 7 shots temp. increased to aprox. 75-80°C (Without cooling with Cryo 6)
Measurement of nail temperature after Nd:YAG laser scanning

Fluence 40 J/cm², Pulsewidth 25msec, Spot size 4mm, Frequency 1Hz.

The nail is fully covered in approximately 15 sec, reaching cca 50°C and the cooling down phase below 40°C is usually longer than 1 minute.
Toenail Nd:YAG laser irradiation

Starting point

5. 6. 7. 8. 9.

10. 11.

Cooling down

The nail is fully covered in approximately 15 sec, reaching cca 50°C and the cooling down phase below 40°C is usually longer than 1 minute.
Candida sp

after 3 months
Aspergilus niger

before

after 6 months
Trichophyton rubrum

before

after 6 months

after 12 months
Trichophyton mentagrophytes

before after 12 months
Trichophyton rubrum

Before after 9 months
Trichophyton rubrum

1.0-1.8 mm/month before after 12 months
Candida sp, Fusarium sp.

Before  

After 3 months  

After 12 months
Trichophyton rubrum

Before

after 6 months
Aspergilus niger

before  

after 12 months
Trichophyton rubrum

Before

after 12 months
Discussion:
Volumetric heat production Nd:YAG
We still have a lot of questions

1. How many treatments do we really need?
2. Mechanism of action?
3. Is it LLLT or not?
4. Is it non ablative laser photodestruction?
5. Is it specific or non specific antifungal therapy?
Laser-tissue interaction

LLLH

Laser beam

Optical Window

Absorbance

wavelength (nm)

water

Hb

HbO2

Melanin

Vaporization

Carbonization

Coagulation

Denaturation

Photothermic effect

Photostimulating effect
Stimulating and positive effects

Inhibitory and negative effects

Biologic effect

The therapeutic window

Dose (J/cm²)

0.01 0.1 1 10 100

Arndt-Schulz law
for an open ulcer:
Doses between 0.1 and 10 J/cm²
are stimulating the
wound healing,
but higher doses
are inhibitory.

35 J/cm²
Nd:YAG laser LLLT

HOST

Positive structural and cellular response.
An increase in cellular migration, viability, proliferation and cytokine expression

Inhibitory effects on cellular migration, a decrease in cellular viability, proliferation and cytokine expression,
An increase of cellular and genetic damage.

Ref: Houreld and Abrahamse 2006

5 J/cm²

16 J/cm²
Time between treatments

- There is no hard and fast rule concerning treatment intervals, but this study demonstrated that appropriate time between treatments was more effective than single irradiation

Adaptive response?

HOST

The first exposure prepares cells for subsequent irradiation
Fungus

- >300 °C  Vaporization, burning
- >150 °C  Carbonisation
- 90-100 °C  Dehydration
- 80 °C  Damage of membrane
- 60-65 °C  Denaturisation of proteine
- 40-45 °C  Formation of edema
- 37-60 °C  Heating, and pretransition in the unfolding of ribonuclease S, due to partial unfolding of the S protein/S peptide complex.

The dissociation occurs at higher temperature.
Mechanism of sequential denaturation means that at least one partial unfolding step comes before the main conformational transition, which is instead a concerted, final unfolding/dissociation step.
Trichophyton mentagrophytes
Laser – Nail tissue interaction

- Due to the light absorbing substance present in fungus, laser irradiation results in excessive heat production which leads to the destruction of the pathogen and therefore to the termination of the infection condition.

- This device employs 1064 nm infrared wavelength that is well known to cause cellular photodamage in the absence of exogenous dyes or other photosensitive drugs and chemicals.
Pigment related photothermolysis

- Lack of growth inhibition in our study might be a supporting evidence regarding pigment-related photothermolysis of T. rubrum rather than inhibition due to nonspecific thermal damage.

- Although the wavelength of Nd:YAG laser at 1,064 nm is beyond the absorption spectrum of xanthomegnin, we have observed inhibitory effects on the colonies treated with this wavelength.

- This might be due to another chromophore absorbing at 1,064 nm, such as melanin, as it is known that Trichophyton species contain melanin in their cell walls.
Heat shock response is nature’s device to protect cells against environmental and physiological stress.

External stresses including heat shock induce the generation of ROS and denaturation of cellular proteins. Activations of signaling pathways in response to a stress vary depending on the strength of stress resulting in the generation of various amounts of ROS and denatured proteins.

Strong stress which is overflowing the rescuing capacity of cells, induce cell death.

Membrane lipid ceramide has been proposed as a signaling molecule that converts extracellular stresses into intracellular signals. In response to heat shock, ceramide levels increased in normal HL-60 cells.
HSP 70 in T. rubrum

- **HSP70** in *Trichophyton rubrum* is already detected and carefully characterised.

- **Reactive oxygen species (ROS)** has recently been suggested as a second messenger generated by growth factors and cytokines, including PDGF, EGF, angiopoietin-1, TNFα, and IL-1 in nonphagocytic cells.
Apoptosis

- Denatured proteins disrupt cellular redox homeostasis and increase ROS levels and **ROS induces protein misfolding**. When misfolded proteins are produced, proteolytic machinery is turned on to remove them.

- Most severe protein denaturation leads apoptosis of fungal cell - a programmed cell death or sometimes cell suicide which plays an important role in a wide variety of normal and pathological processes.
Conclusion

- 1064 nm laser irradiation with the capability of delivering destructive high energy pulses to specific targets with minimized surrounding tissue damage would seem to be well suited for the task of eradicating nail fungus and tinea infection.

- LP Nd:YAG photo-inactivate fungal pathogens to a depth below the nail tissue surface leaving the surrounding tissue intact, using safe energy densities in vitro and in vivo at physiologic temperatures.