Novel Laser Therapy in Treatment of Onychomycosis

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ABSTRACT

A clinical study is presented in which novel approach to use laser for treatment of onychomycosis is described. 72 patients with 194 affected nails were treated with long pulse Nd:YAG laser (Dualis SP, Fotona, Slovenia) at a single clinical site (Dr. Kozarev Dermatology Laser Clinic) over a period of 18 months. Mycotic cultures were taken from all affected nails and various fungal infections were positively diagnosed in all 72 patients. Laser treatment consisted of four sessions with one week interval, during which all infected nails were irradiated with laser beam in a manner that the nail plate was fully covered with laser beam in three consecutive passes. The fluences applied were 35-40 J/cm^2 at laser pulse duration of 35 msec. Temperatures achieved on the surfaces of irradiated nails were measured with thermal imager. Follow up was performed at 3, 6, 9 and 12 months, with mycological check ups at 3 and 6 months. On 3 months follow up 95,8% patients were cleared of all fungal infections. On 3 patients (4,2%) with still present infection the complete procedure was repeated. On 6 and 12 months follow ups all patients (100%) were fully cleared of all fungal infections.
There were no noticeable side effects of the treatment and all patients (even those 3 repeating the procedure) were fully satisfied with treatment. Results of this clinical study lead us to conclude that treatment of fungal nail infections with VSP Nd:YAG 1064 nm laser is an effective and safe therapy for onychomycosis.

Key words: onychomycosis, nails fungal infection, Nd:YAG lasers,

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I. INTRODUCTION

Onychomycosis, a persistent fungal infection of the nail bed, matrix or plate, is the most common nail disorder in adults, accounting for one third of all fungal skin infections and up to 50 percent of all nail diseases [1-3]. Toenails are more affected than fingernails. The causative agents of onychomycosis include dermatophytes (fungi that invade only dead tissues of the skin, nails, or hair), nondermatophyte moulds, and rarely, yeasts of the Candida species [4]. The dermatophytes Trichophyton rubrum and Trichophyton mentagrophytes are the most common causative pathogens of onychomycosis, with T. rubrum responsible for approximately 90 percent of cases [1,2]. The overall prevalence of onychomycosis ranges from 2 to 14 percent, but it increases with age. Fifteen to twenty percent of persons between the ages of 40 and 60 have the condition, compared with 32 percent of persons between ages 60 and 70, and 48 percent of persons who are older than age 70 [1]. Recent evidence suggests that the overall incidence of onychomycosis is increasing [1,2]. Several conditions can mimic onychomycosis, including psoriasis, atopic dermatitis, nail trauma, contact irritants, and lichen planus.

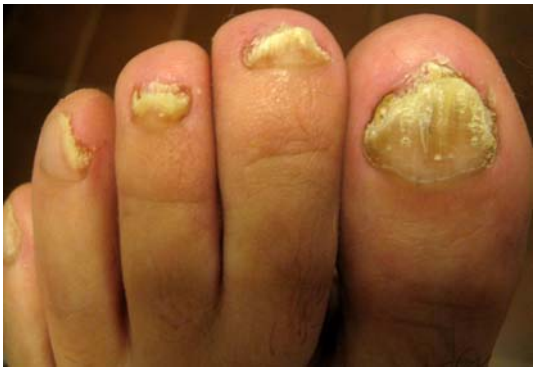

Fig. 1: An example of severe distal (and lateral) subungual onychomycosis

Therapeutic options for the treatment of

onychomycosis range from no therapy, palliative care, mechanical or chemical debridement, topical and systemic antifungal agents to a combination of two or more of these modalities. Factors that influence the choice of therapy include the presentation, and severity of the disease, current medications the patient is taking, previous therapies for onychomycosis and their response, physician and patient preference and the cost of therapy [5]

The treatment of advanced onychomycoses is in general time-consuming and cost-intensive. Even by therapies with potent systemic antimycotics delivered over a period of several months, cure rates of only 40 to 70 percent and 35 to 80% were reported [23-25]. Among the orally delivered systemic drugs Terbinafine, Intraconazole and Fluconoazole are most commonly used. However, systemic drug therapy could be associated with some unpleasant side effects. Headache, rash and gastrointestinal upset, were reported in about 7 percent of patients treated with Intraconazole [23] and about 5 percent of patients treated with Fluconoazole suffered of nausea, headache, pruritus and liver enzyme abnormalities [26]. The duration of systemic drug therapy is usually up to three months, with possibility to reduce it to approximately half with so called "pulse" therapy when increased drug doses are administered.

Topical antifungal preparations are also widely used.. Although safe and relatively inexpensive, topical therapy is seldom effective [27].

The efficacy of the treatment can be improved and its duration reduced by supplementing the medicamentous therapy with some complementary (e.g. light) treatment. Photodynamic therapy has been recently proposed to treat T. rubrum infection, and promising results were obtained [6].

The direct effect of laser light on fungal isolates and affected nails has not yet been rigorously examined for its possible inhibitory potential.

Although there are already two laser system manufacturers promoting their systems for treatment of onychmycosis, none of them had delievered some clinically validated reports of their results so far.

II. MATERIALS AND METHODS

194 nails of 72 patients having clinically and mycologically proven onychomycosis, were submitted to transcutaneous laser irradiation with the aim of deactivation and eradication of fungal infection.

Inclusion study criteria were: toenail fungus and finger nail fungal infection in any of four clinical types of fungal nail infection: total dystrophic form, distal subungual onychomycosis, proximal subungual onychomycosis and endonyx onychomycosis. Ages between 18-45 years old, and all patients signed informed consent before laser procedure.

Exclusion study criteria were: actual systemic antifungal therapy or oral antifungal 6 months before laser procedure, usage of local antifungal therapy such as solutio Castellany, which are changing nails pigmentation. Actual usage of nail coloring dyes or photosensibilisators which are changing nails pigmentation. Pregnancy and children under 12 years of age. Existence of subungual hematoma or nevoid subungual formation, bacterial nail infection which are changing nail pigmentation or existence of concomitant nail disorders such as psoriasis of nail plate, lichen planus and atopic dermatitis.

Primary outcome measures: Clinical improvement Time Frame: 12 months

Secondary outcome measures: Mycological negative Time Frame: 3 months

Special attention was made about factor which cause additional nail pigmentation such as nail polishes which may contain magnesium or iron, intake of isotretinoin or some antimycotic drugs, longer use of griseofulvin or other cefalosporins, minocyclin, local application of cytostatics (busulfan, 5-fluorouracil), professional exposure to dyes and asphalt. Also vasodilators which are increasing the blood flow through the nail region (and thus enable the quicker cooling) have to be exluded.

Treatment procedure

The first step was sample collection process performed thorough cleansing of the nail area with alcohol to remove contaminants. For distal subungual onychomycosis, the infected nail was clipped proximally and the nail bed and underside of the nail plate were scraped with a 1-2 mm serrated curette. For proximal subungual onychomycosis, the normal surface of the nail plate was pared down with a no.15 surgical blade at the lunula and the white debris is collected with a sharp curette from the deeper portion of the plate and the proximal nail bed. The sampled material was divided into two portions: one for direct microscopy and the remainder for culture.

Prior to laser treatment positive fungal cultures were obtained from all patients after direct microscopy as a screening test. Scrapings were mounted for direct examination in 25% KOH mixed with 5% glycerol, heated (e.g., for 1 h at 51 to 54 C) to emulsify lipids, and examined under 3400 magnification for fungal

structures. Culture examination was executed by independent microbiological laboratory (Mikrobioloska laboratorija Paster, Belgrade, Serbia).

Total number of patients included in this study was 76, but 72 concluded it, appearing on all follow-ups.

In order to improve better penetration of laser light through the nail tissue, the treated area of thicker nails were preferably pretreated in order to render their texture open. The patient's total dystrophic form of nails were treated with a preparation containing: Urea 40 % Anhydrous Lanolin 20 % White Wax 5 % White Petrolatum 35 % for three successive nights, by applying the preparation to the nail under occlusion. Such preparation was needed only in three cases.

Treatment was performed using long pulse VSP 1064 nm Nd:YAG laser (Dualis SP; Fotona, Slovenia), with a laser fluences in the range of 35 to 40 J/cm2 , a spot size of 4 mm, and pulse duration of 35 ms. The variations in fluence were selected based on the thickness of the nail to be treated, a thicker nail required a higher fluence. The pulse rate was 1 Hz. Laser beam was applied to substantially the entire nail plate by incrementally moving the beam in spiral pattern as shown on Fig.2. After the entire nail plate was irradiated, the treatment was stopped for 2 minutes and repeated by covering the nail with two more additional passes. The total therapy consisted of four sessions with one week interval between sessions.

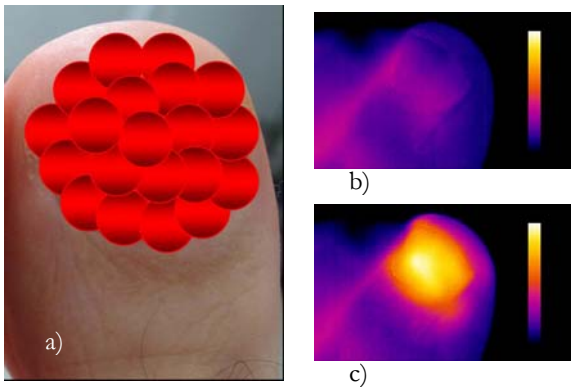

Fig. 2: Presentation of delivery of laser beam in spiral pattern on the nail plate surface (a) Thermal images of toenail surface before (b), and after (c) irradiation with VSP Nd:YAG laser beam. Temperature increase of the nail plate is clearly visible.

For the first few patients the temperature increase of the nail plate was measured during the treatment using FLIR Thermal Imager and ThermaCAM Researcher Pro 2.8 software with which the level of temperature increase was determined (see Fig.2 b) and c) as well as Fig.4).

No local anesthesia was applied preoperatively. The only means to reduce patients discomfort during treatment was cooling with cold air, provided by commercially available air cooling device (Cryo6, Zimmer, Germany).

No postoperative analgesic treatment was required. No prophylactic antibiotics or antiviral were given to any patient.

Parallel to in-vivo therapeutic irradiation of nails infected with fungi an in-vitro experiment of laser irradiation effect on fungus culture was performed.

An isolate of fungal colonies obtained from a toenail scraping were passed on Sabouraud Peptone-Glucose Agar. Ten days after isolation standardized photographs was obtained, the colonies were exposed to laser irradiation with 1064 nm wavelength, fluence of 40 mJ/cm^2 and pulse duration of 35 msec. After two days of laser exposure final colony exam was made and standardized photographs were obtained.

Treatment Evaluation

Follow-ups were done on 3, 6, 9 and 12 months. The patients were objectively evaluated for clearance of fungal infection clinically by physician executing the procedure and mycologically by analysis of the culture taken on 3 and 6 months follow-up visits made by independent microbiological laboratory (Mikrobioloska laboratorija Paster, Belgrade, Serbia). Photographs were taken using the same camera settings, lighting, and treated nails positioning at baseline and at the 6, 9 and 12-month follow-up visits.

Also, all the patients were filling-in the questionnaires after each therapeutical session, evaluating the level of procedural pain (on a 5-point scale where 0 = no pain, 1 = mild pain, 2= moderate pain, 3 = severe pain and 4 = intolerable pain) as well as possible adverse effects, if any of such would occure.

III. RESULTS

a) Types of onychomycosis treated

Treated patients had all four major clinical types of onychomycosis: total dystrophic form, distal subungual onychomycosis, proximal subungual onychomycosis and endonyx onychomycosis. The distribution of onychomycosis types in patient population is given in Table 1.

Table 1: Clinical types of fungal nail infection in treated group.

Type of onychomycosis	Number of patients (%)
Total dystrophic	6 (8.3%)
Distal subungual	38 (52.8%)
Proximal subungual	22 (30.5%)
Endonyx	6 (8.3%)

b) Types of diagnosed fungal infections

As expected, the most frequent fungus found among treated patients was Trichophyton rubrum (in 37 patients or 51,4%), followed by Trichophyton mentagrophytes (22 patient or 30,5%). Table 2 presents all fungi found in patient population.

Table 2: Resultes of fungal nail isolates in a primary isolation medium Sabouraud Peptone-Glucose Agar.

Type of fungal isolates	Number of patients
Candida sp.	10
T. rubrum	37
T. mentagrophytes	22
Aspergilus niger	3

c) Eradication of nail fungal infections

On 3 months follow up 95,83% patients were cleared of all fungal infections. On 3 patients (4,17%) with still present infection the complete procedure was repeated. On 6 and 12 months follow ups all patients (100%) were fully cleared of all fungal infections.

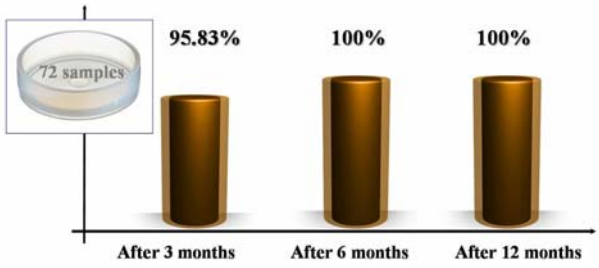

Fig. 3: Efficacy of laser treatment of onychomycosis, as observed from mycological cultures taken on 3 and 6 months and clinically evaluated on 12 months.

d) Temperatures measured on the nail plate

Measurements of the nail plate temperature showed similar behaviour on all tested specimens.

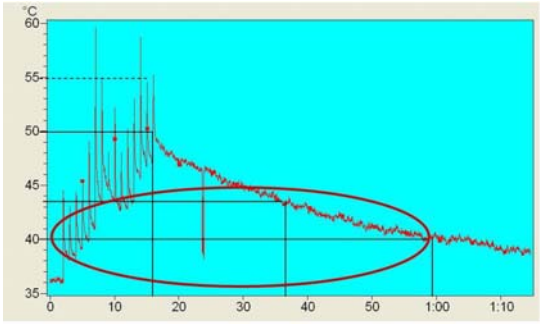

Fig. 4: Measured temperature at the nail plate during the laser treatment.

Working with laser energy delivery rate of 1 Hz and using spot size of 4 mm, the nail plate was fully covred with laser energy in approximately 15 seconds. During that time the temperature in average increased to about 50 °C. After the delivery of energy was discontinued, the nail plate was cooling down, reaching 40 °C in about 1 minute after the beginning of irradiatin.

e) Treatment pain and adverse effect evaluation

Patients were evaluating the treatment pain level after every of four sessions. Their evaluations were averaged and the results are presented in Table 3 below. Most of the patients in average reported mild pain, while none reported severe or intolerable pain.

Table 3: Patients averaged evaluation of treatment pain.

Pain level	No.of patients (%)
(0) No pain	33,33%
(1) Mild pain	48,61%
(2) Moderate pain	18,06%
(3) Severe pain	0,00%
(4) Intolerable pain	0,00%

Many of patients developed a kind of pain resistance during the therapy. Usually they felt the highest level of pain during the first session. On following sessions patients' pain scores were usually lower, as they become "adopted", or they already knew what pain level they could expect.

Patients were also asked to register all potential treatment adverse effects. There were no reports of unwanted side effects resulting from treatments.

f) In-vitro fungus eradication

All in-vitro irradiated samples of fungal cultures were showing evident growth inhibition and colonies decay after single irradiation session. Example of an in-vitro fungal colony development, prior to laser irradiation is shown on Fig.5 a) and its diminishing

after the irradiation is shown on Fig.5 b).

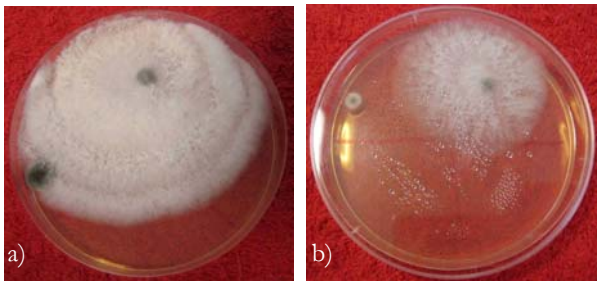

Fig. 5: Petri dish with T. mentagrophytes before and 2 days after long pulse VSP NdYAG laser irradiation.

IV. DISCUSSION

Dermatophyte cells infect skin by a process of adherence to the cells of the epidermis followed by germination, growth, and penetration by fungal hyphae both within and between cells. The first phase of fungal attack on the stratum corneum, the outer layer of cornified cells, dead cells filled with the fibrous protein, keratin, depends on this process of intercellular adherence. Initial studies of this phenomenon utilized microconidia obtained from pure dermatophyte cultures [14]

The principle means of defense against dermatophytes identified at present involve both non-immunological processes such as the interaction between fungi and unsaturated transferrin, activation of epidermal peptides, the inhibitory effect of fatty acids in sebum, and immunological processes including fungal killing by polymorphonuclear leucocytes attracted into the area of infection as well as the activation of T lymphocytes [14].

Increasingly onychomychosis is being viewed as more than a mere cosmetic problem. Persons with unsightly infected nails may suffer embarrassment. Fungi from the nails may precipitate secondary bacterial infections, cellulitis, idiopathic reactions and chronic urticaria. Infected toenails may act as a reservoir for fungi, facilitating their transmission to other areas of the body and even to other people.

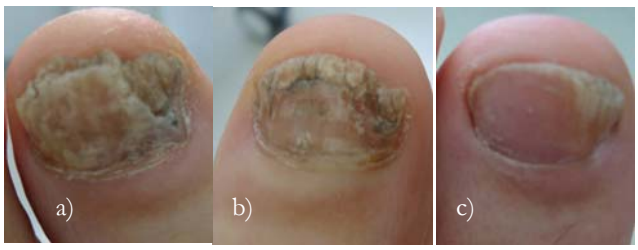

Fig. 6: Trichophyton rubrum treated with VSP Nd:YAG laser: before a), 6 months after b) and 12 months after c)

Clinical diagnosis of onychomycosis is based on the patients' history; a physical examination, microscopy and culture of nail specimens. Predisposing factors like diabetes, old age, hyperhidrosis, onychoglyphores, nail trauma, poor peripheral circulation are likely to be present. Several nail disorders that may mimic fungal nail infections must be correctly recognized and differentiated from onychomycosis to initiate the most appropriate therapy. They include psoriasis, lichen planus, bacterial infections, contact dermatitis, traumatic onychodystrophies, paronychia congenital, nail bed tumors, yellow-nail syndrome, idiopathic onycholysis etc.

There are a lot of factors which are contributing to fungal nail infection such as: diabetes, professional exposure to sugar (cooks, confectioneries, candy makers, sportsmen), exposure to traumas (minor or mayor trauma like subungual hematomas), activities contributing to excessive sweating of feet and skin maceration, visits to pedicure treatments.

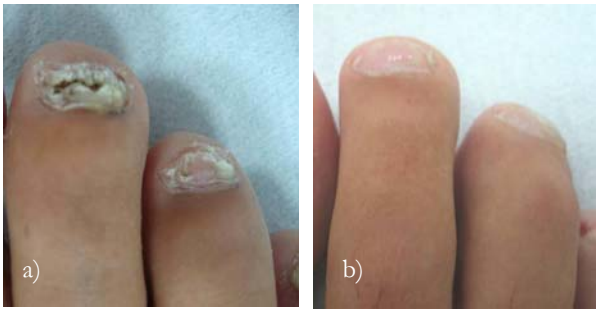

Fig. 7: Trichophyton mentagrophites treated with VSP Nd:YAG laser : before a) and 12 months after b)

Each of the four clinical types of onychomycosis, as defined by the route of fungal invasion, has a characteristic appearance, but other diseases, particularly psoriasis, may have a similar appearance. Proper management, therefore, includes confirmation of fungal infection by potassium hydroxide slide preparation and culture.

Traditionally, pharmacologic treatment has been less than optimal. In many cases, griseofulvin, the first oral agent approved for onychomycosis, must be given for a year or more to be effective. Low cure rates are related to poor bioavailability and the fungistatic rather than fungicidal effect of the drug. Newer agents, such as oral itraconazole and oral terbinafine, promise to substantially increase cure rates while shortening treatment duration. Oral terbinafine is potently fungicidal against dermatophytes and has proven efficacious with regimens as brief as 12 weeks when infection is not spread over the entire nail [23-25].

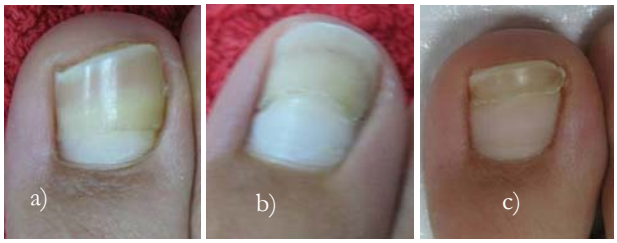

Fig. 8: Candida species treated with VSP Nd:YAG laser: before a), 6 months after b) and 9 months after c)

Success on the clinical usage of lasers largely depends on the wavelength, output power, pulse duration, exposure time, spot size, type, and color of tissue which has to be irradiated [11,12,13].

One of main advantages of laser surgery is its bactericidal effect. Laser light causes local hyperthermia, destruction of pathogenic microorganisms, sanitation of pathology focus and stimulation of reparative process [28].
Statistically significant growth inhibition of T.rubrum was detected in colonies treated with the 1,064-nm Q-switched Nd:YAG laser at 4 and 8 J/cm^2 [29]. This laser produces significant inhibitory effect upon the fungal isolate T.rubrum in in-vitro study. Meral, Tasar at al. reported high bactericidal effect on Candida albicans suspension after NdYAG laser irradiation [30]. These findings suggest that with distinct energy level NdYAG laser has a higher bactericidal effect on smaller Candida population.

The laser used in this study –VSP Nd:YAG 1064 nm, is penetrating through the nail plate and produces heat deep within the dermis and nail tissue.

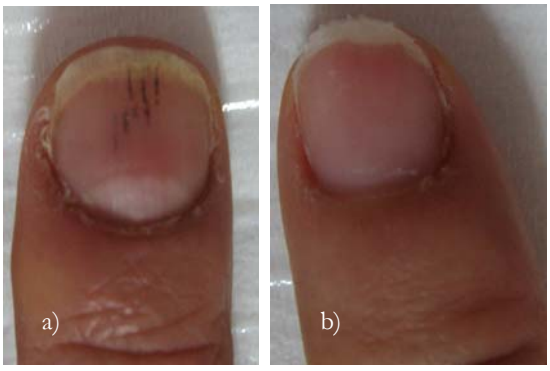

Fig. 9: Aspergilus niger treated with VSP Nd:YAG laser: before a) and 6 months after b)

Melanin is an essential constituent of the fungal cell wall that has been described in many pathogenic species. The type of melanin varies although it is commonly Dopa or pentaketide melanin. Melanized fungal cells show enhanced capacity to resist T-cell mechanisms and neutrophil attack largely through neutralizing the effect of oxidative products such as superoxide or reactive oxygen. Dermatophyte infections are normally eliminated through a largely Th1 path involving effector mechanisms that range from accelerated epidermal turnover to production of adhesion molecule-directed neutrophil trafficking in the epidermis at the site of infection and subsequent phagocyte-mediated fungal cell destruction.

The 1064 nm radiation emitted by the Nd:YAG laser is primarily absorbed by dark pigments. When Nd:YAG laser is used at high power settings and for a long time, the rise in temperature has deleterious effects on periodontal tissues [16]. It is generally agreed that temperatures above 56-60°C cause denaturation of hard tissue proteins [7,8] Eriksson & Albrektsson reported that 47°C temperature for 1 minute (only 10°C above human body temperature) produced persistent bone damage [8]. Levy et al. demonstrated the level of energy is a critical factor to obtain safe treatment conditions [9].

Local hyperthermia is common therapy in Japan in the treatment of sporotrichosis since 1966 with good results [21]. It was applied with infra red and far infra red devices. A big disadvantage of this method was application of non sophisticated devices and necessity of daily applications.

Desired average tissue temperature for laser irradiation of onychomycotic nails is about 43-51.degree. C. at a treatment time of at least 2-3 minutes provides a therapeutic dose. Toleration of higher temperatures is in correlation with possible desensitization of the treated area or increased blood flow.
Amount of laser energy that can deactivate 80-99% of the organisms present in affected nail is deactivating dose. That dose does not instantly kill the fungal colonies but results in their disability to replicate or survive according to apoptotic mechanism. Killing of fungal colonies may be caused by superheating mechanism and exploding or rupture of fungal cells membranes.

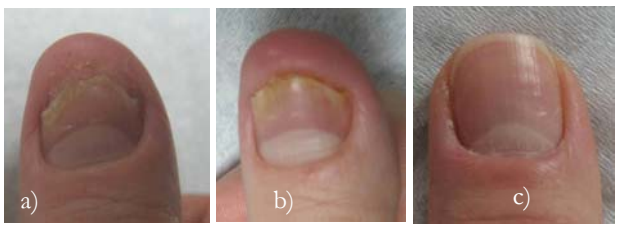

Fig. 10: Candida species treated with VSP Nd:YAG laser :

before a), 3 months after b) and 9 months after c)

Inflammation of the skin and hyperthermia, both of which occur with injury or infection, include a hyperthermic component that many believe constitutes a physical stress. Such increases in local temperature may also have a regulatory effect on immune function. Langerhans cells (LCs), the dendritic cells of the skin, continuously monitor the extracellular matrix of the skin by taking up particles and microbes that they then carry to draining lymph nodes for presentation to T lymphocytes. There are hypothese that the thermal element of inflammation may help regulate the activation and migration of LCs out of the epidermis. These facts support the notion that there may be a beneficial role of hyperthermia. If such increases in temperature are considered a physiologically relevant heat stress, it might be suspected that certen temperatures have the potential to drive an immunomodulatory stress response. This hyperthermia treatment has been shown to be sufficient to induce heat shock protein expression in lymphocytes and other tissues [16,17].

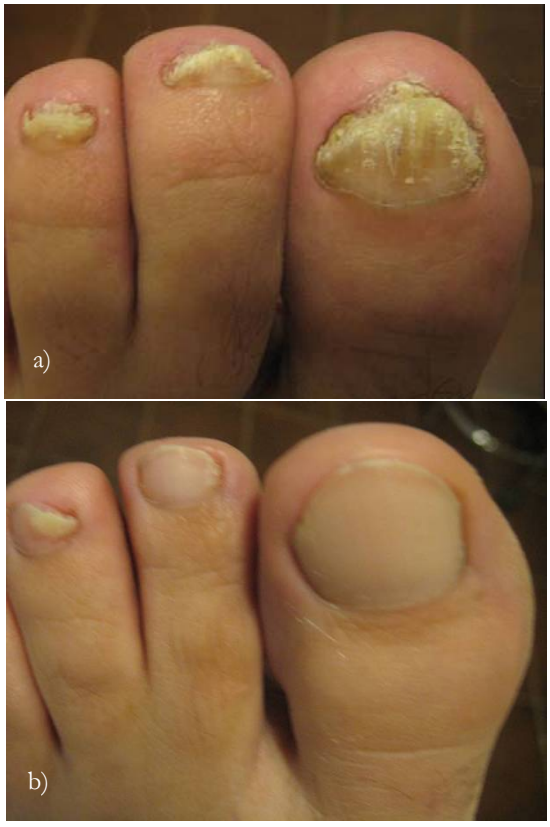

Fig. 11: Trichophyton rubrum treated with VSP Nd:YAG laser: before a) and 12 months after b)

Apoptosis, a physiological type of cell death, plays an important role of selective deletion of cells in divergent situation of various tissues (Levine et al, 1991; White, 1995). The events that are able to induce apoptosis are incredibly diverse but are generally classified into one of three categories: induction by direct DNA damage e.g. strand breaks, chromosomal aberrations, induction bytransduced signals e.g. FAS/APO-1 transmembrane signals,and stress (heat) mediated apoptosis. Hyperthermia, a typical environmental stress, has long been known as toxic to cells. It has been recognized the mode of cell killing to be influenced by severity of the heat treatment [18]. A number of reports have been published to demonstrate the induction of apoptosis by mild hyperthermia [19,20]. Some of the possibilities are that thermal injury may initiate a death signal, target certain heat labile proteins, or cause direct or indirect DNA damage leading to apoptosis. Apoptosis is the result of a combination of the thermal destruction (directly or indirectly) of apoptosis protecting molecules with a concurrent production of killing molecules which then execute the death sentence.
External stresses including heat shock induce the generation of reactive oxygen species (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. HSP70 in Trichophyton rubrum is already detected and carefully characterised. Reactive oxygen species 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. 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 [20,22]
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.

V. CONCLUSIONS

Nd:YAG 1064 nm laser irradiation with the capability of delivering destructive high energy pulses to specific targets with minimized surrounding tissue damage seems to be well suited for the task of eradicating nail fungal infection. This wavelength 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. Reduction of nail plate thickness before laser treatment on severely distrophic nails is enabling optimal effect of NdYAG laser procedure.

The procedure is simple and quick with no noticeable side effects and complications. VSP Nd:YAG laser therapy of onychomycosis is safe and very efficient method for treating all types of onychomycosis caused by various fungal species.

This method is useful for the broadest range of patients and is specially beneficial in elderly, compromised and hepatopathic patients for which other alternative treatments could present some risks.

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