Original Article

Self-controlled Study of Onychomycosis Treated with Long-pulsed Nd:YAG 1064-nm Laser Combined with Itraconazole

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Abstract

Background: Onychomycosis is a fungal infection of the nail plate and subungual area. In this study, we examined the efficacy of laser treatment using self-controlled study programs involving a long-pulsed Nd:YAG 1064-nm laser combined with oral medication.
Methods: Self-controlled strategies were followed in this study. The patients received treatment with oral itraconazole in conjunction with long-pulsed Nd:YAG 1064-nm laser treatment at the nails of the unilateral limb once a week for a total of four times. A total of 84 affected nails were divided into Group A (mild to moderate) and Group B (severe) according to disease severity. Affected nails with the same Scoring Clinical Index for Onychomycosis scores were selected to compare the therapeutic effects of the pure medication treatment group and the combination treatment group with a 24-week follow-up period.
Results: In Group A, at the 8th, 16th, and 24th weeks of follow-up, the efficacies in the pure medication treatment group were 81.0%, 81.0%, and 90.5%, respectively, while those in the combination treatment group were 100%, 95.2%, and 90.5%, respectively. The differences between groups were not significant (8th week: $\chi^2 = 4.421$, $P > 0.05$; 16th week: $\chi^2 = 2.043$, $P > 0.05$; 24th week: $\chi^2 = 0.00$, $P > 0.05$). In Group B, at the 8th, 16th, and 24th weeks of follow-up, the efficacies in the pure medication treatment group were 61.9%, 66.7%, and 52.4%, respectively, while those in the combination treatment group were 95.2%, 90.5%, and 100%, respectively. The differences between groups at the 8th and 24th weeks of follow-up were statistically significant (8th week: $\chi^2 = 6.929$, $P < 0.05$; 24th week: $\chi^2 = 13.125$, $P < 0.05$).
Conclusions: For patients with mild or moderate onychomycosis, we recommended a pure medication treatment or combination treatment with medication and laser. For those patients with severe onychomycosis, we recommended a combination of medication and laser therapy.

Key words: Itraconazole; Laser Treatment; Nd-YAG Laser; Onychomycosis

Introduction

Onychomycosis is a fungal infection of the nail plate and subungual area. The global incidence is relatively high, at around 2–18%.[1] The current main therapy of onychomycosis is topical or oral antifungal medication. Since it is difficult for topical antifungal agents to penetrate the nail plate to the nail bed, the treatment period is long and the efficacy is normally poor.[2] Although the efficacy of oral antifungals is good, its effectiveness is only 60–70%,[3,4] and its potential side effects, such as liver and kidney dysfunctions, restrict its usage.[5] Therefore, it is very difficult to cure onychomycosis.[6] Laser therapy for onychomycosis is a new breakthrough due to its fewer side effects. The number of professional lasers approved by the US Food and Drug Administration is continually increasing, most of which are the long-pulsed Nd:YAG 1064-nm lasers.[7-9] Many researchers believe that the laser treatment of onychomycosis is safe and effective.[10-12] Recently, however, Hollmig *et al*.[13] stated that the use of Nd:YAG 1064-nm laser equipment is invalid for the treatment of onychomycosis. Adopting a self-controlled method, we studied the efficacy of Nd:YAG

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1064-nm laser therapy combined with oral medication as a treatment for onychomycosis.

Methods

Inclusion criteria

The patients were 18–75 years old and had typical clinical manifestations of onychomycosis, including positive fungal direct microscopy and/or fungal culture. The patients signed written informed consent and voluntarily accepted follow-up. None had taken external medication within 1 month or systemic antifungal drugs within the previous 6 months. The affected nails were on the hands or feet, and the severities of bilateral onychomycosis were similar, which meant that the Scoring Clinical Index for Onychomycosis (SCIO) of at least one pair of affected nails of patients with bilateral onychomycosis was consistent.[14]

Exclusion criteria

The following patients were excluded from the study: those who terminated the treatment on their own initiative, changed the treatment strategy, or failed to complete timely follow-up; patients who took other drugs that might affect efficacy during the treatment period; those who persistently or semi-persistently demonstrated nail discoloration, including a nail pigmentation anomaly caused by therapeutic or cosmetic purposes, such as topical antifungal solution castellani, nail stains, polish (such as magnesium- or iron-containing substances), or caused by occupational exposure to dyes or asphalt; those who took a photosensitizer for almost 6 months; pregnant or lactating women; those who had a subungual hematoma or mole-like tissue; those whose affected nail was caused by a combination of diseases, such as nail plate psoriasis, lichen planus, and atopic dermatitis; those with liver or kidney dysfunctions; those who needed to take drugs that cannot be taken in conjunction with itraconazole capsules; and those who were considered ineligible to participate in the trial by clinicians. Treatments terminated due to adverse reactions were not included in the efficacy but were included in the incidence of adverse reactions.

Patient information

Nineteen patients in this study (8 men, 11 women; age range, 19.0–63.0 years; mean age, 45.3 years) were recruited from the Dermatology Department of Beijing Friendship Hospital between October 2013 and March 2014. Their disease duration was 5 months to 25 years (mean duration, 12.2 years). The total number of affected nails was 120, with an average of 6.3 for each patient, and 84 (42 pairs) affected nails met the inclusion criteria.

Grouping

A self-controlled strategy was followed in this study. The patients received oral systemic treatment, and at the same time, laser therapy was conducted on a unilateral limb's affected nails. To minimize differences between the pure medication group and the combination treatment group, the following method was adopted to collect the experimental data: those patients who took oral itraconazole regularly were divided into odd- and even-numbered groups. To do this, twenty tags were labeled with twenty consecutive numbers selected from random number table. For patients in the odd-numbered group, the affected nails of the left limb were subjected to laser treatment. For patients in the even-numbered group, the affected nails of the right limb were subjected to laser treatment. One affected nail from the pure medication group and one from the combination treatment group with the same SCIO score were selected to create a matching pair. Each affected nail was paired only once. Only matched pairs were included in the clinical evaluation and mycological examination.

Matched pairs were divided into two groups (Group A, mild to moderate; Group B, severe) according to disease severity. The SCIO scores of Group A (21 pairs) were $6 \leq$ SCIO <12 while those of Group B (21 pairs) were SCIO ≥ 12. Each group was divided into a combination treatment group (laser combined with medication treatment) and a control group (pure medication treatment).

Therapy

For the laser therapy, a Miracle Laser™ ML-3420 long-pulsed Nd:YAG 1064-nm laser (Wuhan Miracle Laser Co. Ltd., Wuhan, China) treatment device with 5–15 J/cm² fluence, 3-mm spot size, 0.3–2.0-ms pulse width, and 1–10-Hz frequency was used. The energy was adjusted according to the nail thickness: the thicker the nails, the higher the energy. The laser beam irradiated the entire nail plate with a spiral movement and increasing laser energy. After the entire nail plate was irradiated, the process was paused for 2 min, then irradiation was conducted again, and the laser irradiation was repeated three times. Treatments were delivered at 7-day intervals for a total of four times.

For the medication therapy, the antifungal itraconazole (Xi'an Janssen Pharmaceutical Ltd., China) was administered orally. Each treatment course consisted of 200 mg twice daily for 1 week followed by a 3-week rest. Fingernails were treated with three courses while toenails were treated with four courses.

Follow-ups were conducted at the 8th, 16th, and 24th weeks. Fungal microscopy and culture examinations were performed. The patients' adverse reactions, including their duration and extent, were recorded in detail.

Assessment

For the mycological assessment, fungal microscopy and culture examinations were conducted before therapy at the 8th, 16th, and 24th weeks of follow-up. The positive rate = positive/total cases × 100%.

The clinical efficacy assessment consisted of target nail measurements before treatment and at the 8th, 16th, and 24th weeks. Cure was defined as the growth of a new nail with a smooth and brightly colored nail plate and not more than 5% defects. Statistically significant improvement was defined as 60% new nail growth. Improvement was defined as $20\% \geq$ nail growth $<60\%$ while no change

was defined <20% new nail growth. Efficacy rate = cure rate + significant efficacy rate.[10]

Among the twenty enrolled patients, only one patient was lost to follow-up at the early stage due to his migration.

Statistical analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The Chi-square test of paired design was analyzed to compare variables and $P < 0.05$ was considered statistically significant.

Results

Mycological efficacy

In this study, fungal microscopy and culture were conducted on the target nails of each patient who met the inclusion criteria. The microscopic examination results were all positive. Among the 19 patients, fungal cultures were positive in 10 patients (52.6%), including nine cases of *Trichophyton rubrum* (47.4%) and one case of yeast (5.3%). The positive rates of fungal microscopy and culture are listed in Table 1. In Group A, the fungal culture results were significantly different between the combination treatment and control groups at the 8th week ($\chi^2 = 11.667$, $P < 0.05$) but not at the 16th or 24th weeks (16th week: $\chi^2 = 4.286$, $P > 0.05$; 24th week: $\chi^2 = 2.100$, $P > 0.05$). In Group B, the fungal culture results were significantly different between the combination treatment and control groups at the 16th week ($\chi^2 = 11.667$, $P < 0.05$) but not at the 8th or 24th weeks (8th week: $\chi^2 = 0.104$, $P > 0.05$; 24th week: $\chi^2 = 4.421$, $P > 0.05$).

Clinical efficacy

The clinical efficacy of onychomycosis treatment is listed in Table 2. Figures 1 and 2 show different time points of patients' toenail conditions in Group A and B. At the 8th, 16th, and 24th weeks, the difference in clinical efficacy between the combination treatment and control groups in Group A was not statistically significant (8th week: $\chi^2 = 4.421$, $P > 0.05$; 16th week: $\chi^2 = 2.043$, $P > 0.05$; 24th week: $\chi^2 = 0.00$, $P > 0.05$). However, at the 8th and 24th weeks, the difference in clinical efficacy between the combination treatment and control groups in Group B was significant (8th week: $\chi^2 = 6.929$, $P < 0.05$; 24th week: $\chi^2 = 13.125$, $P < 0.05$) while that at the 16th week was not statistically significant ($\chi^2 = 3.535$, $P > 0.05$).

Safety evaluations

No serious adverse events were experienced in any of the 19 patients. Most patients had just a little pain with fine tolerability during the whole treatment.

Discussion

Oral antifungal treatment is considered the gold standard for onychomycosis.[15] In recent years, researchers have been

Table 1: Mycological examination results of affected nails before and after onychomycosis treatment

Time	Group	Positive fungal microscopy, *n*	Positive fungal culture, *n*	χ^2	*P*
Before	Group A				
	Combination	21	13		
	Control	21	14		
	Group B				
	Combination	21	14		
	Control	21	14		
8th week	Group A			11.667	<0.05
	Combination	3	1		
	Control	12	11		
	Group B			0.104	>0.05
	Combination	9	7		
	Control	13	8		
16th week	Group A			4.286	>0.05
	Combination	2	1		
	Control	8	6		
	Group B			11.667	<0.05
	Combination	2	1		
	Control	10	11		
24th week	Group A			2.100	>0.05
	Combination	2	0		
	Control	4	2		
	Group B			4.421	>0.05
	Combination	2	0		
	Control	5	4		

Group A: Mild to moderate onychomycosis severity; Group B: Severe onychomycosis severity.

Table 2: Clinical efficacy of onychomycosis treatment

Group	Cases, *n*	Efficacy rate, *n*		
		8th week	16th week	24th week
Group A				
Combination	21	21	20	19
Control	21	17	17	19
χ^2		4.421	2.043	<0.001
P		>0.05	>0.05	>0.05
Group B				
Combination	21	20	19	21
Control	21	13	14	11
χ^2		6.929	3.535	13.125
P		<0.05	>0.05	<0.05

Group A: Mild to moderate disease severity; Group B: Severe disease severity.

trying to replace medication with laser treatments to improve the cure rate, reduce the incidence of adverse reactions, benefit patients with liver and renal insufficiencies, and avoid drug-resistant pathogens.[16-18] However, to date, laser therapy has not been widely used in clinical practice; hence, the appropriate therapy course and parameters remain under investigation and a number of problems require exploration. Recently, studies by Hollmig *et al*.[13] suggested that Nd:YAG 1064-nm laser equipment is invalid for the treatment of onychomycosis, which has attracted wide attention. The purposes of this study were to determine whether laser treatment for onychomycosis is valid, effective in patients whose conditions are resistant to drug treatment, and better used in combination with medication.

Our *in vitro* studies indicated that the long-pulsed Nd:YAG 1064-nm laser can effectively inhibit the growth of *T. rubrum*.[17] In our previous study, 154 affected nails of 33 patients were randomly divided into two groups, and all cases were treated with long-pulsed Nd:YAG 1064-nm laser. Group 1 consisted of 15 patients (78 affected nails) who underwent therapy once weekly for 8 consecutive weeks. Group 2 consisted of 18 patients (76 affected nails) who underwent therapy once weekly for 4 consecutive weeks and follow-up for 24 weeks. Our results indicate that long-pulsed Nd:YAG 1064-nm laser treatment of onychomycosis is safe and effective as evidenced by the lack of a statistically significant difference between the two groups.[10]

Itraconazole is a broad-spectrum antifungal agent which belongs to the triazole group. Inhibiting the activity of the sterol 14α-demethylase on which the fungal cytochrome P450 relies can interfere with the synthesis of ergosterol, an important component of fungal cell membranes, as well as inhibit fungal growth and promote fungal death.[19] The basic principle of laser treatment is using the selective photothermolysis between the laser and the biological tissue. Recent studies have suggested that the laser band of the Nd:YAG 1064-nm laser is located in the near-infrared band. It can transmit to 3–5 mm under the epidermis and is mainly absorbed by the skin melanin and hemoglobin. When the laser reaches the skin, it is selectively absorbed by the melanin in the fungal cell wall, thus creating the antifungal effect. After being selectively absorbed by the target color base, the laser energy may be converted to vibrational and rotational energy consisting of biological molecules, resulting in enhancement of the thermal motion of the biological molecules and a temperature increase in the irradiated local tissue. The average temperature of the nail plates may increase to 43–51°C, which is enough to cause cell degeneration, subject cellular proteins to oxidative stress, induce cell apoptosis, and effectively kill 80–90% of microorganisms.[4] Since laser therapy and medication have different mechanisms of action, we speculate that combination therapy may be more effective and benefit more patients.

A self-controlled strategy was followed in this study. The patients received oral systemic treatment, and at the same time, laser therapy was conducted in the affected nails of a unilateral limb. Affected nails with the same SCIO scores were selected to compare the efficacy differences between the combination treatment and control groups. The patients were divided into the mild or moderate group (Group A) and severe group (Group B) according to disease severity. In the mild or moderate group, at the 8th, 16th, and 24th weeks, the effectiveness of the combination treatment group was higher than that of the control group; however, the difference between these two groups was not significant ($P > 0.05$). Therefore, combination therapy or medication only can be used in patients with mild or moderate disease. However, in the severe group, at the 8th, 16th, and 24th weeks, the efficacy of the combination treatment was 30–40% higher than that of the medication-only treatment, and the difference between the two groups was statistically significant ($P < 0.05$). In the severe group, the efficacy of the combination therapy was much greater than that of the medication alone. Thus, we believe that the combination treatment is more effective and that the laser treatment helps improve the efficacy of the medication therapy. We recommend that patients with severe onychomycosis can be treated with oral medication as well as laser therapy to improve efficacy and reduce the treatment duration.

At the 8th, 16th, and 24th weeks, the positive rate of the fungal culture of the combination treatment group was lower than that of the pure medication group; however, the overall difference was not significant ($P > 0.05$), which means that the fungal culture result was not consistent with the efficacy results. We believe that the mechanism of laser treatment of onychomycosis is very complicated and that the mycological test results cannot fully reflect the treatment's clinical efficacy. The mechanism of laser treatment not only relies on the laser's direct fungicidal effect but also includes immune system or local microenvironment changes induced by the laser.[20] There was no much difference between the efficacy of the pure medication in this study and that in literature.[21,22] The efficacy of the combination treatment was higher than that of the laser therapy only in the previous research.[1,10,12]

Figure 1: Different time points of a patient's toenail conditions in Group A. Combination therapy side: (a) before treatment and at the (b) 8th, (c) 16th, and (d) 24th weeks. Pure medication therapy side: (e) before treatment and at the (f) 8th, (g) 16th, and (h) 24th weeks.

Figure 2: Different time points of a patient's toenail conditions in Group B. Combination therapy side: (a) before treatment and at the (b) 8th, (c) 16th, and (d) 24th weeks. Pure medication therapy side: (e) before treatment and at the (f) 8th, (g) 16th, and (h) 24th weeks.

In summary, combined laser and medicine treatment for onychomycosis is effective. Laser treatment can improve the efficacy of pure medicine treatment. For patients with severe onychomycosis, we recommend the use of combination treatment. However, further studies are required to understand whether the efficacy of combination treatment is a simple superposition of laser treatment and drug treatment and whether laser irradiation changes the drug's effect on the fungi. This study's primary limitation is its short (4-week) treatment period, which was chosen since laser treatment for onychomycosis is still in the exploratory stage, that might have affected our results.

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Conflicts of interest

There are no conflicts of interest.

References

1. Li Y, Yu S, Xu J, Zhang R, Zhao J. Comparison of the efficacy of long-pulsed Nd:YAG laser intervention for treatment of onychomycosis of toenails or fingernails. J Drugs Dermatol 2014;13:1258-63.
2. Rosen T, Stein Gold LF. Antifungal drugs for onychomycosis: Efficacy, safety, and mechanisms of action. Semin Cutan Med Surg 2016;35 3 Suppl 3:S51-5. doi: 10.12788/j.sder.2016.009.
3. Westerberg DP, Voyack MJ. Onychomycosis: Current trends in diagnosis and treatment. Am Fam Physician 2013;88:762-70.
4. Ortiz AE, Avram MM, Wanner MA. A review of lasers and light for the treatment of onychomycosis. Lasers Surg Med 2014;46:117-24. doi: 10.1002/lsm.22211.
5. Singal A, Khanna D. Onychomycosis: Diagnosis and management. Indian J Dermatol Venereol Leprol 2011;77:659-72. doi: 10.4103/0378-6323.86475.
6. Gupta AK, Studholme C. Novel investigational therapies for onychomycosis: An update. Expert Opin Investig Drugs 2016;25:297-305. doi: 10.1517/13543784.2016.1142529.
7. Gupta A, Simpson F. Device-based therapies for onychomycosis treatment. Skin Therapy Lett 2012;17:4-9.
8. Gupta AK, Simpson FC. Medical devices for the treatment of onychomycosis. Dermatol Ther 2012;25:574-81. doi: 10.1111/j.1529-8019.2012.01519.x.
9. Ledon JA, Savas J, Franca K, Chacon A, Nouri K. Laser and light therapy for onychomycosis: A systematic review. Lasers Med Sci 2014;29:823-9. doi: 10.1007/s10103-012-1232-y.
10. Zhang RN, Wang DK, Zhuo FL, Duan XH, Zhang XY, Zhao JY. Long-pulse Nd:YAG 1064-nm laser treatment for onychomycosis. Chin Med J 2012;125:3288-91. doi: 10.3760/cma.j.issn.0366-6999.2012.18.017.
11. Gupta AK, Simpson FC, Heller DF. The future of lasers in onychomycosis. J Dermatolog Treat 2016;27:167-72. doi: 10.3109/09546634.2015.1066479.
12. Helou J, Maatouk I, Hajjar MA, Moutran R. Evaluation of Nd:YAG laser device efficacy on onychomycosis: A case series of 30 patients. Mycoses 2016;59:7-11. doi: 10.1111/myc.12425.
13. Hollmig ST, Rahman Z, Henderson MT, Rotatori RM, Gladstone H, Tang JY. Lack of efficacy with 1064-nm neodymium:yttrium-aluminum-garnet laser for the treatment of onychomycosis: A randomized, controlled trial. J Am Acad Dermatol 2014;70:911-7. doi: 10.1016/j.jaad.2013.12.024.
14. Sergeev AY, Gupta AK, Sergeev YV. The Scoring clinical index for onychomycosis (SCIO index). Skin Therapy Lett 2002;7 Suppl 1:6-7.
15. Gupta AK, Simpson FC. New pharmacotherapy for the treatment of onychomycosis: An update. Expert Opin Pharmacother 2015;16:227-36. doi: 10.1517/14656566.2015.993380.
16. Wanitphakdeedecha R, Thanomkitti K, Bunyaratavej S, Manuskiatti W. Efficacy and safety of 1064-nm Nd:YAG laser in treatment of onychomycosis. J Dermatolog Treat 2016;27:75-9. doi: 10.3109/09546634.2015.1034078.
17. Xu ZL, Xu J, Zhuo FL, Wang L, Xu W, Xu Y, *et al*. Effects of laser irradiation on *Trichophyton rubrum* growth and ultrastructure. Chin Med J 2012;125:3697-700. doi: 10.3760/cma.j.issn.0366-6999.2012.20.018.
18. Gupta AK, Paquet M. Management of onychomycosis in Canada in 2014. J Cutan Med Surg 2015;19:260-73. doi: 10.2310/7750.2014.14090.
19. Shibata S, Takahashi H, Ono N, Wada N, Kubo H, Shinozaki K, *et al.* Longitudinal monitoring of CYP3A activity in patients receiving 3 cycles of itraconazole pulse therapy for onychomycosis. J Clin Pharm Ther 2014;39:181-5. doi: 10.1111/jcpt.12127.
20. Nenoff P, Grunewald S, Paasch U. Laser therapy of onychomycosis. J Dtsch Dermatol Ges 2014;12:33-8. doi: 10.1111/ddg.12251.
21. Pajaziti L, Vasili E. Treatment of onychomycosis – A clinical study. Med Arch 2015;69:173-6. doi: 10.5455/medarh.2015.69.173-176.
22. Gupta AK, Daigle D, Foley KA. Network meta-analysis of onychomycosis treatments. Skin Appendage Disord 2015;1:74-81. doi: 10.1159/000433473.