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Research Article

Evaluation of Efficacy and Safety of Topical Nanoliposomal Amphotericin B 0.4% Gel as a Potential Treatment for Onychomycosis: An Interventional Pilot Clinical Study

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Onychomycosis is a frequent fungal nail disease that is hard to treat and, in most cases, needs long-term therapy with oral agents. Traditionally, oral agents are favored over topical agents, but it should not be overlooked that they come with broad adverse effects and concomitant drug interactions, which can be unsuitable for many individuals. Therefore, alternative approaches need to be addressed by the medical team for numerous cases. On the other hand, local administration of antimicrobials can come as advantageous because of having a more selective site of activity, avoiding off-target systemic adverse effects, and rapid administration at the site of infection. In this study, we are evaluating a new topical delivery method of amphotericin B. To investigate the efficacy and safety of topical nanoliposomal amphotericin B 0.4% as a possible therapeutic option, this pilot, single-group, before-after clinical study was conducted on 15 onychomycosis patients. The evaluation was processed during 36 weeks of follow-up and on three endpoints of week 12, week 24, and week 36, for both clinical and mycological responses. Three patients were excluded; of the remaining 12, 50% showed a complete cure, 16.66% had an effective clinical response, 16.66% had a partial clinical response, and 16.66% showed no response at week 12. Mycological cure was calculated as 50% at week 12. At week 24, our measurements were calculated as 91.66% for complete cure, 8.33% for no response, and 91.66% for mycological cure. One patient reported nail plate detachment at week 2 but continued the topical application; follow-up between weeks 2 and 24 showed a complete cure and regrowth of healthy nails. No other adverse effects were detected. Overall, our study suggests that topical nanoliposomal amphotericin B 0.4% is an effective treatment, which is accessible, affordable, and user-friendly, has minimum adverse effects, and could be regarded as an alternative treatment for those ineligible for systemic therapy. This trial is registered with IRCT20150101020514N18.

1. Introduction

Onychomycosis is an umbrella term commonly used to describe all fungal nail infections [1]. It is responsible for more than 50% of nail disorders. Onychomycosis generally affects 5.5% of the population worldwide and has an international spread [2]. It is the most common nail complaint at dermatology clinics. Age of more than 70 years, immune deficiency, and diabetes are some of the known risk factors for developing onychomycosis [3–5].

Furthermore, diabetic patients are 2.77 times more likely to develop onychomycosis compared to control [6]; nevertheless, the disease can present itself at younger ages and among people with normal immune system status. Clinical presentation of onychomycosis usually follows an interdigital palmar and/or plantar fungal infection that has

progressed to nailbeds. Dermatophyte fungi, non-dermatophyte filamentous fungi, and yeasts are the most common causes of onychomycosis [1]. *Trichophyton*, *Epidermophyton*, and *Microsporum* among dermatophyte species (spp.); *Aspergillus* and *Fusarium* among non-dermatophyte filamentous spp.; and *Candida*, *Malassezia*, and *Trichosporon* among yeast spp. are the leading pathogens causing onychomycosis [1]. Clinically speaking, *Trichophyton rubrum* and *Trichophyton interdigitale* from the dermatophyte family are the main responsible fungi for the majority of the cases.

Several systemic and topical medications are available for the treatment of onychomycosis. Traditionally, oral agents are favored over topical agents by clinicians. The reason behind this is that topical therapy normally has a lower diffusion rate into the nailbeds and hence takes more time to reach a favorable clinical response. Terbinafine, itraconazole, and fluconazole are some of the available oral agents. Among those patients who have completed their treatment cycle, these agents have shown response rates of 76%, 58%, and 48% in clinical studies, respectively [7, 8]. Oral itraconazole or terbinafine are traditionally recommended as the first-line therapy by clinicians [8]; nonetheless, it should not be overlooked that due to their broad adverse effects and concomitant drug interactions, both are unsuitable for many individuals. Therefore, alternative approaches need to be addressed by the medical team for numerous cases [9]. Multiple topical agents are also at the disposal, such as ciclopirox olamine, clotrimazole, and efinaconazole. Despite their conventional lower potencies and more limited on-site penetration, local administration of antimicrobials can come as advantageous because of having a more selective site of activity, avoiding off-target systemic adverse effects and rapid administration at the site of infection. Furthermore, the recent progress made in the field of drug delivery such as novel molecular configurations for the drug or its vector (e.g., liposomal forms), or local laser-based drug delivery with fractional ablative lasers to increase the absorption of topical treatments [10], has offered new perspectives for clinicians to reconsider topical therapies. Overall, the efficacy of current therapies varies in different individuals and independent studies that are conducted at different times [7], possibly due to recent fungal drug resistance and nonselective general treatments that fail to target the causative fungi. Therefore, onychomycosis seems to have become more frequent than any time before. These observations alarm the need for finding novel and more efficient therapeutic approaches for the treatment of onychomycosis that are suitable and affordable for all patients while keeping the unwanted systemic adverse effects to the minimum [7]. This study aims at evaluating the efficacy of a newly manufactured topical agent (topical nanoliposomal amphotericin B 0.4%) as a potential treatment for onychomycosis in the setting of a pilot clinical trial study.

Amphotericin B is an antibiotic produced by *Streptomyces nodosus* [11] and has been used for a long time for the treatment of systemic fungal infections [12]. Its mechanism of action mostly relies on pore formation in the fungal plasma membrane, thus inducing cell death by osmotic lysis. Systemic administration of amphotericin B can come with certain adverse effects, including nephrotoxicity, discomfort at the site of injection, and constitutional symptoms and therefore should be prescribed selectively with prior precaution and indication [13]. Liposomal amphotericin B (AmBisome®), lipid complex amphotericin B (Abelcet®), and colloidal dispersal of amphotericin B are the current available lipid-based formulations of this drug [14].

Nanoliposomal forms of amphotericin B are novel formulations of this drug with minimal toxic effects. They are capable of passing natural biological skin barriers by targeting cutaneous macrophage cells in the dermis and epidermis [15]. Topical nanoliposomal amphotericin B 0.4% is one of the recently available agents of this group. It has a size of approximately 100 nm and its efficiency has been evaluated as promising for the treatment of cutaneous leishmaniasis in previous studies [16]. An *in vitro* study of topical nanoliposomal amphotericin B 0.4% for the treatment of fungal infection has shown compelling results for two clinically important dermatophytes (*T. rubrum* and *T. interdigitale* (recently renamed as *T. indotineae*)) [17], yet the drug has not been clinically assessed among patients. The purpose of this study is to evaluate the efficacy of topical nanoliposomal amphotericin B 0.4% for the treatment of onychomycosis in a clinical pilot study. We pharmaceutically produced topical nanoliposomal amphotericin B 0.4% gel (SinaAmpholeish® 0.4%) at Minoo Company in Tehran, Iran, for the upcoming administration in the clinical setting [18].

Specifically, the investigators of this study wish to (I) determine the safety and efficacy of this therapeutic approach for the treatment of onychomycosis, (II) determine the required treatment duration to cure the nails and eliminate the fungus, and (III) report posttreatment findings including treatment failure, reinfection, or recurrence.

2. Materials and Methods

2.1. Study Design and Ethical Statement. The study was conducted from December 2021 to October 2022 as a pilot, single-group, before-after study with a sample size of 15 patients who were affected by onychomycosis. Treatment was continued for a period of 12–36 weeks with a primary endpoint follow-up at week (W) 36. Before the study, all patients had a private visit with the medical team in which all of their medical questions were addressed and a full medical and drug history was obtained, including any history of previous antifungal therapy. Key inclusion and exclusion criteria are defined in Table 1.

A consent form was later given to the clinically eligible people to be read and signed. All patients agreed to the study protocol and were fully aware of the therapeutic process and were informed about all of their other treatment options. This research was permitted by the Iranian Registry of Clinical Trials, with reference no. IRCT20150101020514N18.

Table 1: Inclusion and exclusion criteria.

(A) Key inclusion criteria
Male or female subjects of any race, 18 to 60 years of age (inclusive)
Verbal and written informed consent/assent obtained from the subject
Good general health, as assessed by the investigator, based on the subject's medical history, physical examination, and safety laboratory tests
Target nails for all subjects must have had evidence of nail growth, per subject's report that monthly clipping is needed
Subjects are willing to comply with study instructions and return to the vising clinic for all required appointments every 12 weeks for at least 3 visits
(B) Key exclusion criteria
Male or female who have received oral/IV antifungal therapy within the past 12 weeks prior to screening
If topical antifungal medication was used in the past four weeks prior to testing
Patients who had a history of immunosuppression or clinical evidence indicating possible immunosuppression
Uncontrolled diabetics
Patients who have performed a surgical intervention for nail dystrophy in the past
Any illness or condition that could have caused nail anomalies or adversely affected the assessment, or the presence of any nail infection other than onychomycosis or in addition to onychomycosis
Patients who had received immunosuppressive therapy in the past 3 months prior to screening visit or who had the need for it
Females who are pregnant, nursing a child, or planning a pregnancy during the study duration

2.2. Subjects. In the beginning, this study included 15 subjects (males: 6 individuals and females: 9 individuals) with an age range of 18–60 years. Patients were entered into the study regardless of their underlying causative fungus and there were patients with either dermatophyte, non-dermatophyte, or yeast infection. Onychomycosis was diagnosed clinically and paraclinically using medical assessment along with mycological testing. Fungal nail infection was defined as at least one fingernail or toenail involvement that has been confirmed via direct microscopic examination and positive culture.

2.3. Intervention. Following diagnosis confirmation by the medical team, eligible patients were prescribed 0.4% nanoliposomal amphotericin B gel. They were asked to apply the gel topically twice daily on the entire surface of the affected nails and a 6 mm margin around the cuticle. The time anticipated to reach clinical response was predicted to be 12 weeks for fingernails and 36 weeks for toenails. After the primary visit, 3 subsequent visits were arranged for patients at weeks 12, 24, and 36, respectively.

2.4. Assessment. The primary assessment was to evaluate the efficacy of nanoliposomal amphotericin B 0.4% gel for the treatment of infected nails based on a comparison of clinical and mycological status before and after receiving the treatment at weeks 12, 24, and 36. On each session, patients were clinically examined at the center by a dermatologist, and their data were recorded in the registry. A visual analog scale (VAS) was used in each session to evaluate patient satisfaction. VAS is a numerical (1–10 score) psychometric satisfaction questionnaire. Patients were then referred to do laboratory tests including direct microscopic potassium hydroxide (KOH) smear and culture every 12 weeks. Furthermore, a photograph was taken of the status of the infected nails at each of the times of the visit to record the gradual possible effects of the therapy. The findings of each session were subclassified into 2 major groups:

(i) Mycological assessment scale (MAS), based on the microscopic diagnosis of mycologist: this includes four grades of mild, moderate, severe, and mycological cure (MC). MC is defined as negative direct KOH smear microscopy plus negative culture.

(ii) Clinical response, based on the physical examination report of the dermatologist: this includes (1) partial clinical response (PCR), defined as a visible improvement of nailbed involvement which still affects 10%–50% of the target nail plate, and (2) effective clinical response (ECR), defined as a visible improvement of nailbed involvement that affects less than 10% of the target nail plate.

Eventually, complete cure (CC) is defined as total infection-free nail plate + MC.

2.5. Safety Evaluation. Dryness, scaling, erythema, allergic inflammatory reactions, burning, pruritus, and some other local adverse effects have been previously reported with the usage of topical antifungal medications. Electrolyte disturbance (potassium and magnesium), anemia, nausea, diarrhea, allergic rash, nephrotoxicity, headaches, and constitutional symptoms have been reported with systemic intravenous therapies with amphotericin B.

The probable transdermal distribution of amphotericin B into blood circulation has been previously ruled out by Van Bocxlaer et al. [19]. The assumed distribution volume of liposomal amphotericin B is reported to be 0.1–0.44 L/kg. The maximum therapeutic index of this medication is approximately 1–1.5 mg/kg [20]. Liposomal formulation of amphotericin B contains 4 mg of active

ingredient per 1 gr of the gel. Assuming that the used amount of the gel at each time of application would be approximately 0.5 gr, there would be a sum of 2 mg of the active ingredient of amphotericin B in each dose of treatment. Considering the apparent volume of distribution, the drug concentration in the plasma of a 70 kg individual will be between 0.06 and 0.28 mg/L, which is considerably below the maximum therapeutic level for this agent (2.26–10 mg/L). All these suggest that it seems unlikely for the topical form of amphotericin B to lead to any of the systemic adverse effects of the injectable form. Consequently, it was estimated that systemic side effects such as nephrotoxicity and those related to higher injectable dosages of amphotericin B would be less likely to happen. Nevertheless, all the patients were monitored for any possibility of drug reactions.

2.6. Antifungal Susceptibility Testing. All isolates were tested for antifungal susceptibility to terbinafine, itraconazole, fluconazole, and voriconazole (all reagents from Sigma-Aldrich) based on the broth microdilution method and the CLSI-M38 3rd ed guideline [21]. Minimum inhibitory concentrations (MICs) were determined using the visual examination of fungal growth after 24–72 hours according to the fungal species by an expert mycologist. The *Candida parapsilosis* ATCC 22019 was used as the quality control.

2.7. Statistical Methods. Percentage and frequency were used to explain qualitative records. Furthermore, the MIC range and MIC 50 were calculated. All the statistical calculations were conducted using the IBM SPSS® application, version 22.0.

3. Results

3.1. Participant Demographic Data. Fifteen patients (9 females (60%) and 6 males (40%)) were included in the study. The demographic data and clinical characteristics of these 15 patients are shown in Table 2.

The average age was 45.06 years. The most common site of infection was in the feet (60%), and 53.33% of the participants had more than one infected nail. Distal lateral subungual onychomycosis (DLSO) was the most frequent clinical form ($N = 12/15$, 80%), followed by white superficial onychomycosis (WSO) ($N = 2/15$, 13.33%) and proximal subungual onychomycosis (PSO) ($N = 1/15$, 6.66%). There were no cases of endonyx onychomycosis. 60% ($N = 9/15$) of patients had less than 50% nail plate involvement, 33.33% had an approximate 50% nail plate involvement ($N = 5/15$), and finally, one patient had total dystrophic nail ($N = 1/15$, 6.66%).

3.2. Initial Antifungal Susceptibility Test Results. Table 3 summarizes the MIC ranges and the MIC 50 for six antifungal agents against all the fungal species isolated from the subjects. These included (I) nanoliposomal amphotericin B, (II) conventional amphotericin B, (III) fluconazole, (IV) voriconazole, (V) itraconazole, and (VI) terbinafine. Also, the nanoliposomal carrier was assessed separately in the MIC panel to investigate its independent or synergistic antifungal activity.

Table 2: Demography of onychomycosis cases.

Characteristics	Number of patients (%)
Gender	
Male	6/15 (40)
Female	9/15 (60)
Age groups	
<20	—
20–29	1/15 (6.6)
30–39	4/15 (26.66)
40–49	1/15 (6.6)
50–59	7/15 (46.66)
60–69	2/15 (13.33)
Site of the affected nail	
Fingernails	5/15 (20)
Toenails	7/15 (60)
Fingernails and toenails	3/15 (20)
Risk factor	
Nail manipulation	7/15 (46.66)
Trauma	8/15 (53.33)
Length of involvement	
<1 year	8/15 (53.33)
≥1 year	7/15 (46.66)

3.3. Early Efficiency Endpoint (W12). Two participants were excluded from the study at week 12; one of which had become pregnant and the other one had a renal failure exacerbation and a complementary need for a kidney transplant, which was irrelevant to this clinical trial. One was later excluded at W24 and was not included in the analysis. Of the remaining 12 patients, 6 had CC (50%), 2 had ECR (16.66%), 2 had PCR (16.66%), and 2 had no response (NR) (16.66%) at W12. MC was evaluated as 50% ($N = 6/12$) (Table 4).

3.4. Secondary Efficiency Endpoint (W24-W36). At W24, our measurements were evaluated as 91.66% CC ($N = 11/12$), 8.33% NR ($N = 1/12$), and 91.66% MC ($N = 11/12$). All patients with PCR and ECR in the previous appointment had reached the therapeutic milestone (CC) at W24. Monitoring detected a lack of therapeutic progress in one of the patients. This patient was a female infected with *Fusarium solani* fungus and further medical investigation found out that the subject used nail polish during the therapy and that lack of compliance despite patient education was the main reason behind treatment failure. The patient was excluded from the study at week 24 and was not included in the analysis. There was another patient who did not show any therapeutic response in the clinical setting or the mycological evaluation until the end of the study period (W36). This patient had an infection with *Candida glabrata* and more than 50% nail plate involvement at the beginning of the study. He was eventually considered the only NR patient (Table 4).

Table 3: MIC range and MIC 50 of antifungals against fungal species isolated in this study.

Species (*N*)	MIC	L-C-AMB (μg/mL)	AMB (μg/mL)	L-C (μg/mL)	VCZ (μg/mL)	ITZ (μg/mL)	FCZ (μg/mL)
C. albicans (*N*: 7)	MICs range	0.03–0.0626	0.03–0.5	0.03–0.25	0.03–0.5	0.03–1	0.125–4
	MIC 50	0.03	0.03	0.03	0.25	0.5	0.25
C. glabrata (*N*: 4)	MICs range	0.03–0.125	0.03–0.25	0.03–0.5	0.03–0.5	0.03–0.5	32
	MIC 50	0.03	0.03	0.5	0.03	0.125	32
T. rubrum (*N*: 2)	MICs range	0.03–0.125	0.03–0.5	0.03–0.125	0.03–0.25	0.03–0.125	32
	MIC 50	ND	0.03	ND	ND	ND	ND
F. solani (*N*: 2)	MICs range	0.03	0.125	0.03	0.25	0.0625	32
	MIC 50	ND	ND	ND	ND	ND	ND

AMB: amphotericin B; L-C: lipid conjugate; L-C, liposomal carrier; VCZ, voriconazole; ITZ , itraconazole; FCZ, fluconazole; MICs, minimum inhibitory concentrations; ND, not determined.

3.5. Summary of Results and Report of Adverse Effects. A chronological record of patients' therapeutic progress and response rates is summarized in Table 4. Out of 15 included subjects at the beginning of the study, two participants were excluded at W12; one due to pregnancy (patient ID: 15) and the other due to renal failure which was found to be unrelated to this experiment (patient ID: 2). One participant (patient ID: 11) was excluded at W24 due to noncompliance. The remaining 12 patients continued the trial till the end of the study.

A yellowish-to-brownish change in the color of the target nails was the most common complaint of this study, starting at W4 on the site of the topical application. At W12 (first follow-up appointment), all of the 12 actively participating patients had reported a change in nail color, which was also detected by the medical team during the examination. 2 of the patients that had experienced this discoloration at the beginning of W4 reported that the color change started to resolve after a while and was fully reversed by W12. There was medical documentation of this progress via the sent photographs of the patients to the medical team. One patient reported nail plate detachment at W2 but continued the topical application of the drug on the nailbed until W24, and further follow-up of the patient between W2 and W24 showed CC and regrowth of healthy nails. No other adverse effect was detected throughout this study. Figures 1–3 illustrate the clinical response of onychomycosis before and after treatment with topical nanoliposomal amphotericin B 0.4%.

4. Discussion

This study represents an interim report of the first assessment of the safety and efficacy of topical nanoliposomal amphotericin B 0.4 for the treatment of onychomycosis and demonstrates effective therapeutic results with minimal adverse effects and no relapse rates. The defined cure milestones of this study were 12 weeks of treatment for fingernail infection and 24 weeks of treatment for toenail infection, which were achieved for 11/12 of the participants.

This experiment included subjects with a broad spectrum of clinical presentations of onychomycosis, including different sites of infection and three distinguished clinical subclasses of onychomycosis (DLSO, PSO, and WSO), all of which had an efficient response to the therapy (Table 4). 6 of the cases were older than 50 years of age. Given the prior knowledge that older age is related to probable less efficient blood circulation in small vessels, such as those in nails, it is assumed that a clinical response might take longer time due to slower suppliance for nail regrowth. Yet all of these patients (except for one (patient ID: 8) whose case is discussed in detail later in this section) also reached the therapy milestones as anticipated (fingernail at 12 weeks and toenail at 24 weeks) as well as other participants.

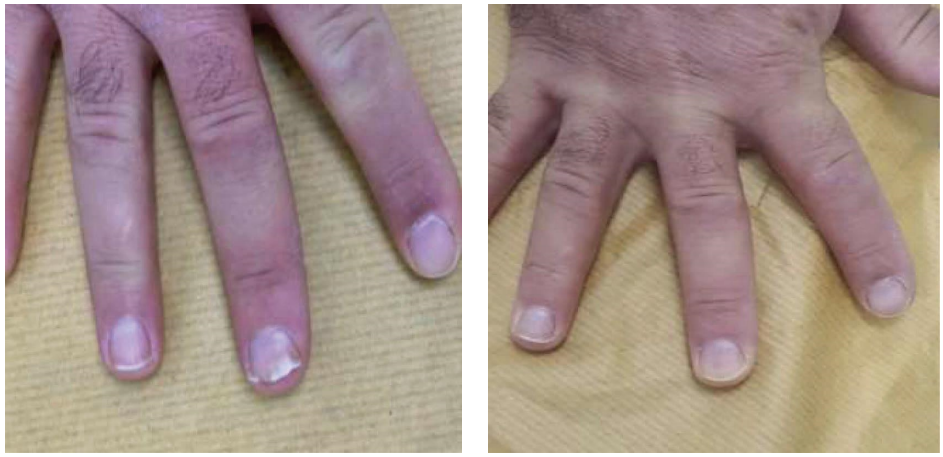
There are two previous studies about the usage of topical amphotericin B for the treatment of onychomycosis, one with amphotericin B in 30% dimethyl sulfoxide and the other with 0.2% topical amphotericin B in 50% dimethyl sulfoxide. Both of these had promising results with MC of 80% (8/10) plus the clinical cure of 70% (7/10) over 72 weeks for the first study and MC of 87.5% (7/8) over 52 weeks for the latter [22, 23]. To our knowledge, this is the first clinical trial that has evaluated the nanoliposomal form of amphotericin B for the treatment of onychomycosis.

At W12 of this trial, MC and CC were both calculated as 50% (CC: 1/6 toenail (TN), 4/6 fingernail (FN), and 1/6 TN + FN) (Table 4). We had one patient with a toenail infection (patient ID: 4) that reached CC at W12 which was earlier than anticipated (W36); this patient had *C. albicans* DLSO. There was also another subject with mixed TN and FN infection that was completely cured at W12 (patient ID: 1). We deduced that this early response is related to higher penetration levels of the liposomal form medication that infiltrates the dorsal nail plate easier and advances mycological clearance and that patients with less involvement are more likely to reach the therapeutic goal sooner than assumed as both of these cases had less than 10% nail plate involvement on each of the target nails. There was a considerable increase in MC (91.66%) and CC (91.66%) at W24 compared to the ones at W12. All of the patients with PCR and ECR at W12 reached CC by W24, suggesting that longer durations of treatment (24 weeks) might come with better therapeutic response rates and that the results would continue to increase over time. We had one patient (patient ID: 4) who had FN DLSO with 20% nail involvement and was resistant to the previous oral therapy with fluconazole. Furthermore, she had a MIC greater than 1 mg/mL for fluconazole, confirming resistance as the reason for her previous treatment failure. She reached CC with nanoliposomal amphotericin B 0.4% at W12 with no report of prominent side effects, which according to the recent reports of antifungal resistance can be accounted as a promising result to consider. One of the subjects (patient ID: 8)

Table 4: Fungal species, history of antifungal treatment, and outcome of topical nanoliposomal amphotericin B 0.4% in onychomycosis patients.

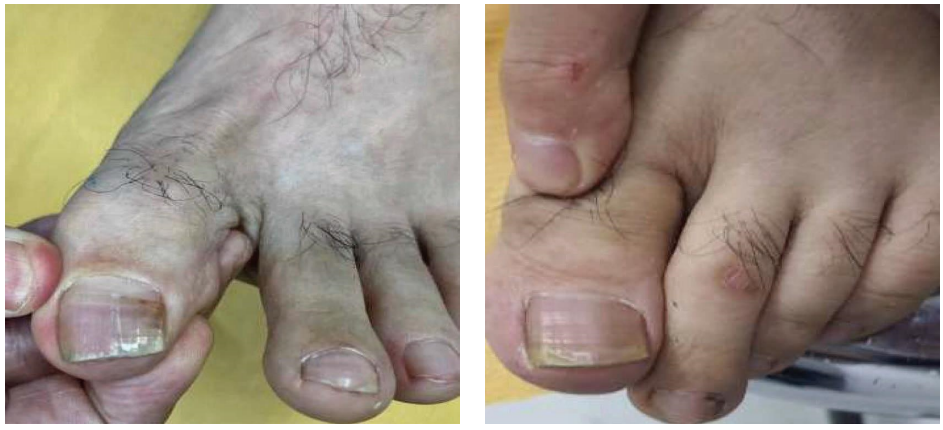
	Patient ID	Fungal species	DH	Site of infection	Type of OM	Nail plate involvement (%)	MAS							AE	
							MILD	MOD	SEV	MC	PCR	ECR	CC	NDC	ND
F/50	1	*C. albicans*	NU	FN + TN	DLSO	<10				W12			W12		
F/58	2	*C. glabrata*	NU	TN	DLSO	50			W12				E/W24	W12	
F/20	3	*T. rubrum*	Fluconazole	FN	WSO	50	W12			W24	W12		W24	W12	
F/37	4	*C. albicans*	Clotrimazole-fluconazole	FN	DLSO	20				W12			W12	W12	
F/35	5	*C. albicans*	NU	TN	DLSO	30	W12			W24			W24	W12	
F/30	6	*C. albicans*	Clotrimazole	TN	DLSO	10				W12			W12	W12	
F/34	7	*C. albicans*	NU	Big TN	DLSO	50	W12			W24	W12		W24		W12
M/54	8	*C. glabrata*	Fluconazole	Big TN	DLSO	50<				NC at W36	W36		NC at W36	W12	
F/50	9	*C. albicans*	Clotrimazole	FN + TN	DLSO	20	W12			W24		W12	W24	W12	
M/60	10	*C. glabrata*	NU	FN + TN	DLSO	30		W12		W24		W12	W24	W12	
M/60	11	*F. solani*	NU	TN	DLSO	50			W24				E/W12	W12	
M/45	12	*C. glabrata*	NU	FN	DLSO	10				W12			W12	W12	
M/51	13	*C. albicans*	NU	FN	DLSO	20				W12			W12	W12	
M/59	14	*T. rubrum*	NU	FN	WSO	10				W12			W12	W12	
F/33	15	*F. solani*	Fluconazole	TN	PSO	50							E/W12	W12	

DH, drug history; OM, onychomycosis; MAS, mycological assessment scale; MOD, moderate; SEV, severe; PCRP, partial clinical response; ECR, effective clinical response; CC, complete cure; AE, adverse effects; NDC, nail discoloration, ND, nail detachment; NU, not used; DLSO, distal lateral subungual onychomycosis; WSO, white superficial onychomycosis; PSO, proximal subungual onychomycosis; W, week. NC: no cure.



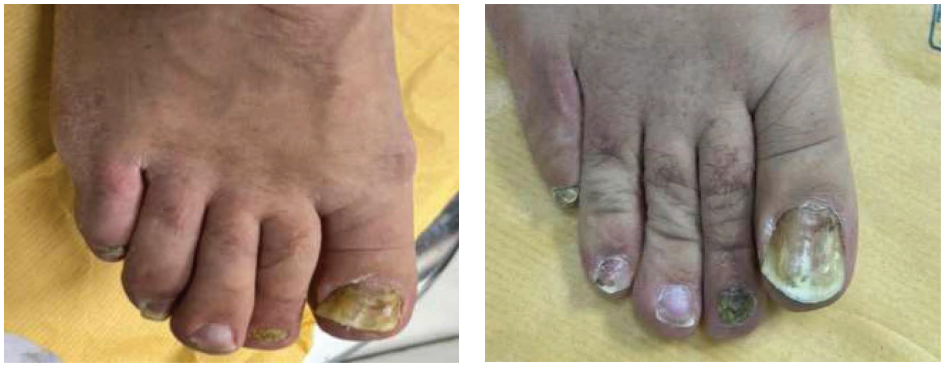
(a) (b)

Figure 1: Complete cure with topical nanoliposomal amphotericin B 0.4% gel (patient no. 12). (a) Before treatment (area affected by *C. glabrata*). (b) After treatment (cured W12).



(a) (b)

Figure 2: Complete cure with topical nanoliposomal amphotericin B 0.4% gel (patient no. 14). (a) Before treatment (area affected by *T. rubrum*). (b) After treatment (cured W12).



(a) (b)

Figure 3: (a) Representative photograph of patient no. 8 at baseline (area affected by *C. glabrata*). (b) Representative photographs of clinical failure with topical nanoliposomal amphotericin B 0.4% gel.

did not reach the therapeutic milestone till the end of the study at W36. He was a 54-year-old man with no recorded past medical history of apparent cardiovascular disease nor any other condition that affects nail reperfusion and the healing process. He had previously received oral fluconazole (150 mg once daily for 6 months) but had little to poor response to the treatment. His medical evaluation identified *C. glabrata* DLSO in his big toe with over 50% nail plate involvement (this patient had the highest surface involvement among our subjects). Mycological and clinical evaluations at W12, W24, and the final appointment at W36 showed no progress. However, he mentioned slight improvement via correspondence with the medical team post-W36, although there is no official documentation of this progress. We attributed this poor response to multiple reasons: first, this patient had more than 50% surface involvement, and second, the comparison between the findings obtained from the MIC test (MIC: 0.125 in sessile cells, considered as sensitive) and the clinical outcome (unresponsive) demonstrates that there is a possible additional resistance mechanism in the clinical setting possibly due to biofilm formation of *C. glabrata*. Except for nail discoloration, we found no other general adverse effects in this study. Compared to the side effects of other treatments with the same efficacy that come with more prominent unwanted off-target systemic effects, we can say that nail discoloration can be a rather acceptable outcome. Some of the patients reported a normal color recovery after a while; nonetheless, the duration of this study was not long enough to evaluate whether this adverse effect is reversible or not. All subjects were followed up to 6 months after complete cure with nanoliposomal amphotericin B 0.4%. There was no relapse.

To better evaluate our clinical results, we decided to also test nanoliposomal amphotericin in an *in vitro* setting with an antifungal susceptibility test. The fungicidal activity of nanoliposomal amphotericin B 0.4% is compared with conventional antifungal agents (voriconazole, itraconazole, fluconazole, and conventional amphotericin B) and nanoliposomal vehicle in Table 3. Primarily, the MIC experiment demonstrated that the nanoliposomal vehicle has an independent antifungal activity (MIC range of 0.03–0.25 compared to itraconazole MIC range of 0.03–1 for *C. albicans*, and MIC range of 0.03–0.5 compared to itraconazole MIC range of 0.03–0.5 for *C. glabrata*. Itraconazole is considered the standard control treatment). Also, it was found that amphotericin B and the liposomal vehicle have a synergistic therapeutic effect since MIC range and MIC 50 were more ideal in the case of nanoliposomal amphotericin B compound compared to each of the compounds separately (Table 3). Furthermore, nanoliposomal amphotericin B had lower MIC ranges than other oral medications in all species (Table 3), suggesting that it is potentially a quasi-efficient alternative that demands lower doses and that comes in a topical form without the off-target systemic adverse effects.

5. Conclusions

Overall, our study suggests that topical nanoliposomal amphotericin B 0.4% is an effective treatment, which is accessible, affordable, and user-friendly and comes with minimum adverse effects and it could be regarded as an alternative treatment for patients who are ineligible for systemic treatment.

5.1. Study Limitations. This was a clinical pilot study and our main goal was to evaluate the preliminary outcomes of this novel format of amphotericin B for the treatment of onychomycosis; however, we had limitations to compare and comment on the effect of the drug for different subtypes of onychomycosis, due to small sample size and lack of randomization. Another limitation of this study was the targeted species; we had two cases that were affected by nondermatophyte molds (*F. solani*). Unfortunately, both of these cases were excluded during the task of study: one due to pregnancy and the other due to medical emergency, respectively, which restricted us from drug assessment among nondermatophyte subtypes. To achieve both of these goals, future studies with randomized larger sample sizes and several arms should be defined.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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