Study of Clinically Suspected Onychomycosis in a Podiatric Population

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Onychomycosis, by definition, is a mycotic infection of the keratinized tissue of the nail plate. Although it is commonly considered to be caused by one of the dermatotropic fungi, a variety of other organisms have been implicated as etiologic agents in the disease, including some bacteria and yeasts. When it is caused by a fungus, any or all of three types of organisms can be involved: dermatophytes, yeasts, and nondermatophyte organisms. The purpose of this study was to identify the microorganisms found in fungal cultures of clinically suspected onychomycosis in the patient population of the Foot Clinics of New York in New York City, the largest foot clinic in the world. Of the 1,800 medical charts reviewed, 214 had culture results, of which 120 were positive. *Trichophyton rubrum* was the most prevalent pathogen, found in 67% of positive cultures. The most remarkable risk factor was age, with 80% of affected individuals older than 35 years. False-negatives may account for the high percentage (44%) of negative cultures in this study. (J Am Podiatr Med Assoc 92(6): 327-330, 2002)

Onychomycosis, by definition, is a mycotic infection of the keratinized tissue of the nail plate.[1] Onychomycosis accounts for approximately 30% of all fungal infections and is the most frequently encountered nail disease.[2] The US Health and Nutrition Examination Study of more than 20,000 subjects aged 1 to 74 years found a prevalence of 2.2% for onychomycosis.[3]

Although onychomycosis is commonly considered to be caused by one of the dermatotropic fungi, a variety of other organisms have been implicated as etiologic agents in the disease, including some bacteria and yeasts.[4-6] When onychomycosis is caused by a fungus, any or all of three types of organisms can be involved: dermatophytes, yeasts, and nondermatophyte organisms.[7] The etiology of onychomycosis seems to differ by geographic location. The dermatophytes, in particular *Trichophyton rubrum*, are most frequently seen in the United States,[8] whereas *Candida* has been reported with high frequency in Belgium[9] and Spain.[10] Of 165 positive cultures examined in Hong Kong for single infections, 44.7% were *T rubrum* and 26.3% were *Candida* (other than *Candida albicans*).[11] A Canadian study[12] of 131 patients with mycologically confirmed pedal onychomycosis found that the causative pathogens were predominantly (92.9%) dermatophytes—68% *T rubrum* and 29% *Trichophyton mentagrophytes*. *Scytalidium*, a nondermatophyte, is more frequent in tropical climates.[13] In Britain, *T rubrum* was the most frequent pathologic agent, with *T mentagrophytes* and *Epi-*

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dermophyton floccosum being less common.[14] In eastern Saudi Arabia, one study[15] revealed *Candida* to be the pathogen in 204 of 243 cases of culturally positive onychomycosis.

There are striking differences in the frequency with which each group of fungi is responsible for the mycotic infection.[7] Distal and lateral subungual onychomycosis, which is an infection of the nail bed and plate, is often caused by *T rubrum*, but it may also be caused by *T mentagrophytes* and *E floccosum*. Distal and lateral subungual onychomycosis is the most frequent type of onychomycosis. Once the hyponychium is infected, the fungi advance to the nail bed epithelium. The fungi are sheltered by the nail plate, creating a moist and soft subungual environment ideal for fungus. Once the pathogen invades the matrix, the fungus is entrapped in the newly formed nail and infiltrates the entire nail plate.[2] Proximal subungual onychomycosis is an infection of the proximal nail fold (the eponychium), with the infection extending distally under the nail plate. *Trichophyton rubrum* is most frequently implicated in proximal subungual onychomycosis.[1, 2] *Trichophyton megninii*, *Trichophyton tonsurans*, *Trichophyton schoenleinii*, *T mentagrophytes*, and *E floccosum* have also been reported to cause proximal subungual onychomycosis.[1] White superficial onychomycosis, which is found in toenails only, affects the nail plate and is usually caused by *T mentagrophytes*, which is better suited biochemically to invade the nail plate.[8] White superficial onychomycosis can be caused by nondermatophyte molds such as *Aspergillus* species, *Acremonium* species, *Fusarium oxysporum*, and *Scopulariopsis brevicaulis*.[2, 16]

Although onychomycosis is rare in children, its incidence increases with age.[12] Studies[2, 12] have shown a higher prevalence in males than in females. It is hypothesized that occupational factors may play a role in sex prevalence.[12] Psoriasis has been shown to increase the risk of developing onychomycosis.[17] The consensus is that there is probably not a higher prevalence of onychomycosis in the diabetic nail, but increased *Candida* infections of the nail and surrounding tissues can pose a serious risk in the diabetic patient.[18]

The diagnosis of onychomycosis is made through clinical observation, direct microscopic examination with potassium hydroxide (KOH), fungal culture, and biopsy with histologic analysis. The clinical signs of tinea unguium include thickening subungual debris, discoloration, and surface irregularity. Examination with KOH is a screening tool for the presence or absence of fungus, but it does not identify the organism.

Sabouraud's dextrose agar, with the antibacterial agent chloramphenicol and the mold inhibitor cycloheximide, is used to aid in the identification of fungal pathogens. Histologic analysis evaluates the topographic distribution, density, and nature of fungi. The histologic preparation can be used to confirm or negate the culture results.[19]

The purpose of this study was to identify the microorganisms found in fungal cultures of clinically suspected onychomycosis in the patient population of the Foot Clinics of New York in New York City, the largest foot clinic in the world. This was a systematic, retrospective, epidemiologic survey of onychomycosis based on laboratory data. To the authors' knowledge, these data have not been collected in the past. Other data, such as KOH results, patient demographic information, and incidence of other systemic diseases, were also collected.

Materials and Methods

Eighteen hundred medical charts from patients at the Foot Clinics of New York who had a clinical diagnosis of onychomycosis were randomly chosen and reviewed. Nail clippings, subungual debris, and scrapings were taken from patients clinically suspected of having onychomycosis. The nail samples were carefully transported to the laboratory. Nail portions were taken for clearing with 10% KOH and direct microscopic examination. Nail cultures were inoculated at 30°C and were examined weekly for 4 weeks. The laboratory used Sabouraud's dextrose agar containing cycloheximide (0.5 mg/mL) and chloramphenicol (0.05 mg/mL). Charts with a culture report had pertinent data extracted. All data were documented on the case report forms, including patient demographic information (age, race, and sex) and culture results.

Results

Table 1 lists the species of possible infective organisms from positive cultures of patients with a clinical diagnosis of onychomycosis. Of the 1,800 charts reviewed, 214 had culture results, of which 120 were positive. *Trichophyton rubrum* was the most prevalent pathogen (67%). Eighty percent of affected individuals were older than 35 years. Of 214 patients, 20% were younger than 35 years, 39% were aged 35 to 55 years, and 41% were older than 55 years; 61% were female; and 14% were diabetic. Twenty-eight percent (59/214) of the patients clinically diagnosed as having onychomycosis had negative KOH results. Forty-four percent (94/214) of the fungal cultures were negative. Twenty-one percent of patients with onychomycosis (from whom a culture was taken) had concurrent

Table 1. Fungal Organisms Found in 120 Positive Cultures from Patients with Onychomycosis

Organism	Number (%)
Dermatophytes	
Trichophyton rubrum	80 (67)
Trichophyton mentagrophytes	2 (2)
Yeasts	
Candida albicans	2 (2)
Candida parapsilosis	8 (7)
Rhodotorula rubra	8 (7)
Trichosporon beigelii	2 (2)
Cryptococcus uniguttulatus	1 (1)
Hyalohyphomycetes (nonpigmented fungi)	
Penicillium species	3 (3)
Aspergillus species	3 (3)
Scopulariopsis brevicaulis	1 (1)
Paecilomyces species	1 (1)
Fusarium species	1 (1)
Phaeohyphomycetes (pigmented fungi)	
Aureobasidium pullulans	2 (2)
Chaetomium species	1 (1)
Cladosporium species	1 (1)
Zygomycetes	
Rhizopus species	1 (1)
Mixed infection	
T rubrum, C parapsilosis	2 (2)
T rubrum, Aspergillus niger	1 (1)

tinea pedis. Twenty-nine percent (35/120) of the patients with positive cultures had concurrent tinea pedis, whereas 11% (10/94) of the patients with negative cultures had concurrent tinea pedis.

Five types of molds were isolated from nine patients: *Fusarium* (n = 1), *Aspergillus* (n = 3), *S brevicaulis* (n = 1), *Paecilomyces* (n = 1), and *Penicillium* (n = 3) (Table 1). As no serial cultures were available, these nondermatophyte molds are listed for purposes of information and the reporting of organism distribution. Without serial cultures, it cannot be determined whether they are pathogens or contaminants.[20] Eight percent of the positive cultures showed *Candida*, with 80% (8/10) of those with *Candida parapsilosis* and 20% (2/10) with *C albicans*. Dermatophyte onychomycosis affected more male patients, whereas all ten patients with *Candida* onychomycosis were female.

A study by Ghannoum et al[20] of 253 onychomycotic nail samples showed dermatophytes as the most commonly isolated fungi (59%). A specimen was considered positive if septate hyphae were seen on microscopic examination, culture, or both. Nondermatophyte molds and yeasts represented approximately 20% each. As in the present study, Ghannoum et al found that *T rubrum* was the most common isolate among the dermatophytes. Ghannoum et al, however, found *Acremonium* to be the most common nondermatophyte; the present study showed *Penicillium* and *Aspergillus* to be the most common nondermatophytes. In the study by Ghannoum et al, *C parapsilosis* represented 66.7% of the yeast species, whereas the present study showed *C parapsilosis* at 38%, the same percentage as for *Rhodotorula rubra*.

In a similar study by Srinivas et al[21] in 1993, nail scrapings and clippings were collected from 100 patients. The culture positivity for molds was 22%, with *Aspergillus* species predominating. The criteria for reporting the mold as a pathogen were KOH positivity plus an isolation of the same fungus in culture on three consecutive occasions at intervals of at least 7 days each and absence of systemic or local antifungal treatment during the period of investigation.

Discussion

Onychomycosis, a fungal infection of the toenails and fingernails, can affect standing, walking, and exercising, resulting in pain and discomfort. A definitive diagnosis is crucial for effective treatment because other dermatologic disorders mimic onychomycosis. The easiest and quickest way to confirm the diagnosis is with KOH preparation, in which spores and fungal filaments are easily detected.[2] Mycologic culture is a generally accepted method of confirming the diagnosis.

The yield of positive cultures can be disappointing, as indicated by this study, in which 56% (120/214) of the cultures were positive. Often, not enough specimen is inoculated onto culture media. Therefore, it is important to curette into the "spongy" area to increase the chance of obtaining a positive culture. When interpreting negative culture results, false-negatives are frequent, which may account for the high percentage (44%) of negative cultures in this study. Negative cultures are mostly caused by lack of living fungi in the collection of subungual debris. When a dermatophyte and a nondermatophyte are grown, the dermatophyte is considered the pathogen, although both may play a role in the clinical picture.[2] Further studies warrant serial cultures and microscopic findings for yeast cells as definitive proof that nondermatophyte molds and yeasts are causative pathogens rather than contaminants.

The results of this study show the increasing numbers of nondermatophyte molds and yeasts present in nail infection (*Aspergillus*, 3%; *C parapsilosis*, 7%). *Candida albicans* is usually secondary to chronic paronychia and is found more frequently in finger-

nails, whereas *C parapsilosis* is a less active pathogen and is found mainly in toenails. The nondermatophyte fungi may reflect colonization of an already damaged nail, since dermatophytes are probably the only primary nail invaders. It is important to note, however, the presence of nondermatophyte molds, yeasts, and mixed infections when considering treatment. For future culture analysis, it may be wise to use two culture plates—one with and one without cycloheximide.[2] Cycloheximide-containing cultures do not allow for the recovery of fungi such as *Hendersonula* and *Scytalidium*, which are capable of causing onychomycosis, tinea pedis, and tinea palmaris.[8]

Onychomycosis occurs more frequently in males than in females. This study, however, shows a preponderance of females (61%). The patient population of the Foot Clinics of New York is 62% female and 38% male, which accounts for the increased incidence in females in this study.

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References

1. Lundeen GW, Lundeen RO: Onychomycosis: its classification, pathophysiology and etiology. JAPA **68:** 395, 1978.
2. Haneke E: Fungal infections of the nail. Semin Dermatol **10:** 41, 1991.
3. Prevalence, morbidity and cost of dermatological diseases [editorial]. J Invest Dermatol **73:** 395, 1979.
4. Abramson C, Wilton J: Nail dust aerosols from onychomycotic toenails: part II. Clinical and serological aspects. JAMA **75:** 631, 1985.
5. Taplin D: Superficial mycoses. J Invest Dermatol **67:** 177, 1976.
6. Freund PA: Onychomycosis: diagnosis and treatment. JAPA **64:** 421, 1974.
7. Velez A, Linares MJ, Fernandez-Roldan JC, et al: Study of onychomycosis in Cordoba, Spain: prevailing fungi and pattern of infection. Mycopathologia **137:** 1, 1997.
8. Zaias N: "Onychomycosis," in *The Nail in Health and Disease*, 2nd Ed, ed by N Zaias, p 87, Appleton & Lange, Norwalk, CT, 1990.
9. Achten G, Wanet-Rouard J: Onychomycoses in the laboratory. Mykosen Suppl **1:** 125, 1978.
10. Jimenez Aguirre D, Martinez LM, Pimentel AJ, et al: Estudio de las onicomicosis en el area del poniente almeriense. Actas Dermosifiliogr **85:** 407, 1994.
11. Kam KM, Au WF, Wong PU, et al: Onychomycosis in Hong Kong. Int J Dermatol **36:** 757, 1997.
12. Gupta AK, Jain HC, Lynde CW, et al: Prevalence and epidemiology of unsuspected onychomycosis in patients visiting dermatologists' offices in Ontario, Canada: a multicenter survey of 2001 patients. Int J Dermatol **36:** 783, 1997.
13. Elewski B, Greer DL: *Hendersonula toruloidea* and *Scytalidium hyalinum*: review and update. Arch Dermatol **127:** 1041, 1991.
14. Williams HC: The epidemiology of onychomycosis in Britain. Br J Dermatol **129:** 101, 1993.
15. Al-Sogair SM, Moawad MK, Al-Humaidan YM: Fungal infection as a cause of skin disease in the eastern province of Saudi Arabia: prevailing fungi and pattern of infection. Mycosis **34:** 333, 1991.
16. Gupta AK, Elewski BE: Nondermatophyte causes of onychomycosis and superficial mycosis. Curr Top Med Mycol **79:** 87, 1996.
17. Gupta AK, Lynde CW, Jain HC, et al: A higher prevalence of onychomycosis in psoriatics compared with non-psoriatics: a multicentre study. Br J Dermatol **136:** 786, 1997.
18. Rich P: Special patient populations: onychomycosis in the diabetic patient. J Am Acad Dermatol **35:** 510, 1996.
19. Pierard GE, Arrese JE, DeDoncker P, et al: Present and potential diagnostic techniques in onychomycosis. J Am Acad Dermatol **34:** 273, 1996.
20. Ghannoum MA, Hajjeh RA, Scher R, et al: A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. J Am Acad Dermatol **43:** 641, 2000.
21. Srinivas CR, Ramani A, Girija Ratna Kumari T, et al: Molds in onychomycosis. Int J Dermatol **32:** 877, 1993.