Mycopathologia (2005) 160: 125–128
DOI: 10.1007/s11046-005-6872-z

Non-dermatophytic molds as agents of onychomycosis in Izmir, Turkey – a prospective study

S. Hilmioğlu-Polat[1], D.Y. Metin[1], R. İnci[1], T. Dereli[2], I. Kılınç[2] & E. Tümbay[1]
[1]*Departments of Microbiology & Clinical Microbiology, Mycology Laboratory, Faculty of Medicine, Ege University, Izmir, Turkey;* [2]*Departments of Dermatology, Faculty of Medicine, Ege University, Izmir, Turkey*

Received 6 January 2005; accepted in revised form 3 May 2005

Abstract

The purpose of this study was to determine the prevalence of causative non-dermatophytic filamentous fungi in onychomycosis. Totally 1,222 (1,222 × 3 = 3,666) samples of nail scrapings from 1,146 patients (from 76 patients two specimens: both from finger- and toe-nails) with prediagnosis of onychomycosis sent to the Mycology Laboratory from the Clinic of Dermatology, Ege University Hospital, Izmir, Turkey, July 2001–December 2003, were prospectively studied with conventional mycological procedures. The set criteria for the diagnosis of onychomycosis due to non-dermatophytic molds were: (1) Observation of fungal elements in 15% KOH-preparations made from nail scrapings, (2) growth of the same mold in all three consecutive cultures of the specimens taken three times from the same patient with one-week intervals, (3) no growth of a dermatophyte or yeast in three consecutive cultures. As agents of onychomycosis molds were detected in 33 (9%), dermatophytes in 175 (48%), yeasts in 150 (41%), and mixed (two different fungi) in 8 (2%) patients. In cases of mold onychomycosis, 11 (33%) had finger-nail and 22 (67%) toe-nail infection; 25 (76%) were female and 8 (24%) male; and 27 (82%) were above 40 years of age. The agents of mold onychomycosis, in order of frequency, were *Aspergillus niger* (7), *Acremonium* spp. (6), *Fusarium* spp. (6), *Ulocladium* spp. (4), sterile mycelia (2), *Alternaria* sp. (1), *Aspergillus flavus* (1), *Aspergillus fumigatus* (1), *Aspergillus terreus* (1), *Cladosporium* sp. (1), *Paecilomyces* spp. (1), *Scopulariopsis* sp. (1) and *Trichoderma* sp. (1). In conclusion, this study showed that non-dermatophytic molds were responsible for nearly 10% of onychomycoses cases attending the dermatology outpatient clinic of a university hospital in Izmir, Turkey. Since molds are common contaminants in the laboratory, cultures from consecutively taken nail scrapings should be made and carefully evaluated in order to diagnose a ''mold onychomycosis''.

Key words: diagnosis, epidemiology, Izmir – Turkey, molds, onychomycosis

Introduction

Onychomycosis is a commonly encountered superficial infection. Beside causative dermatophytes and yeasts, present data show that non-dermatophytic filamentous fungi can also be the potential cause of this ungual disease [1–11]. Onychomycoses caused by molds are not well understood. Since molds are common laboratory contaminants, medical attention is needed for the identification and evaluation of these potential pathogens, a process which is not always easy.

This prospective study was undertaken in order to determine the prevalence of non-dermatophytic molds as causative agents in onychomycosis.

Materials and methods

The study was conducted in the Mycology Laboratory, Faculty of Medicine, Ege University, Izmir, between dates July 2001 and December 2003. A total of 3,666 samples of nail scrapings from 1,146 (725 females and 421 males) immuno-

competent patients with presumptive onychomycosis was prospectively investigated for the presence of causative fungi [from 245 patients finger-nail (735 samples), from 825 patients toe-nail (2475 samples) and from 76 patients both finger- and toe-nail samples (456 samples)].

The criteria for the diagnosis of onychomycosis due to non-dermatophytic molds were: (1) Observation of fungal elements in the 15% KOH-preparation made from nail scrapings, (2) growth of the same non-dermatophytic mold in all three consecutive cultures of the specimen taken three times from the same patient with 1-week intervals, and (3) no growth of a dermatophyte or yeast in three consecutive cultures (in case of simultaneous growth of dermatophytes or yeasts along with molds, molds not accepted as causative agents).

According to the criteria above, all three consecutive cultures of the specimens taken three times from the same patient with 1-week intervals were reported as one specimen. Thus, all three consecutive cultures of the specimens taken three times from the same patient with 1-week intervals were expressed as one specimen in the tables and text.

Conventional mycological procedures were used for the examination of the samples, namely: 15% KOH-preparations, cultures on Sabouraud dextrose agar (Sigma), potato dextrose agar (Sigma) and mycobiotic agar (Sigma) slants (with and without cycloheximide and antibacterial antibiotics) (for each patient 6 slants each with 3 inoculations) incubated at 26 °C for 4 weeks, culture control twice a week, and identification of grown fungi.

Results

From 366 culture-positive specimens 374 strains of fungi were obtained; 33 (9%) yielded non-dermatophytic molds, 175 (48%) dermatophytes, 150 (41%) yeasts, and 8 (2%) mixed (two different fungi) growth (Table 1). All patients with mold onychomycosis had subungual hyperkeratosis the microscopy of which showed septate-hyphae.

In case of mold onychomycosis; the toe-nails were more frequently affected (in 22/66%) than finger-nails (in 11/34%). The infection was more prevalent in females (in 25/76%) than in males (in 8/24%). Of the cases 27 (82%) were over 40 years of age. The youngest patient was a 5-year-old boy and the oldest case was a 82-year-old female, both affected in toe-nails.

The most frequently isolated four molds were *Aspergillus niger* (in 7/22%), *Acremonium* spp. (in 6/18%), *Fusarium* spp. (in 6/18%), and *Ulocladium* spp. (in 4/12%) (Table 2).

Although all three consecutive cultures of the specimens taken three times from one patient (18/F) with 1-week intervals yielded *Alternaria* sp., the culture was accepted as negative due to the negative result of direct microscopic examination of the specimens (Criterion 1). In all three consecutive cultures from two patients (one from both finger and toe-nail, the other from only toe-nail), fungal elements were observed in the 15% KOH-preparation made from nail scrapings, but upon growth of a yeast and non-dermatophyte mold in culture, these two cases were also not included in results (Criterion 3).

Discussion

Since molds are common contaminants on the skin as well as in the laboratories, for the definitive diagnosis of mold onychomycosis consecutive cultures from the same lesions should be made. Pure growth of the same mold on all consecutive cultures indicates mold onychomycosis [5, 9, 12]. In the present study, the diagnosis of the infection

Table 1. Onychomycosis due to different fungal groups: number of cases and isolated strains

Onychomycosis	Mold	Dermatophytes	Yeast	Mixed (D + Y)	Total cases	Total strains
Finger	11	12	93	3 (**6** strains)	119	122
Toe	22	163	57	5 (**10** strains)	247	252
Total	33	175	150	8 (16 strains)	366	374
	(9%)	(48%)	(41%)	(2%)	(100%)	

D: Dermatophyte, Y: Yeast.

Table 2. Non-dermatophytic molds isolated as agents of onychomycosis

Molds	*n* (%)
Aspergillus niger	7 (22)
Acremonium spp.	6 (18)
Fusarium spp.	6 (18)
Ulocladium spp.	4 (12)
Sterile mycelia	2 (6)
Alternaria sp.	1 (3)
Aspergillus flavus	1 (3)
Aspergillus fumigatus	1 (3)
Aspergillus terreus	1 (3)
Cladosporium sp.	1 (3)
Paecilomyces sp.	1 (3)
Scopulariopsis sp.	1 (3)
Trichoderma sp.	1 (3)
Total	33

was reached according to this criterion. The study reflected that 9% of all mycologically confirmed cases of ungual mycosis were due to non-dermatophytic filamentous fungi (Table 1). Frequencies of mold onychomycosis in European countries like Austria [4], Estonia [11], Italy [9] and Spain [7] are reported as about 5% (mean), 7, 8 and 17.2%, respectively. The prevalences in North America are 4.3% in Canada [6] and 20% in the United States [10], whereas in South America 4.5 and 9.5% in two different centers in Colombia [1] and 1% in Argentinia [13]. In Asia the frequencies are given as 12% in Singapore [2] and 22% in India [3]. A study on onychomycosis from Turkey [14] reports the prevalence as 2.1%, but the study is retrospective with no repeat cultures carried out. The prevalences might change with the study of larger population of patients and with consecutive cultures from the same patient.

Reports show that mold onychomycosis is more frequent in toe-nails than in finger-nails and in the adult age, especially over 50 [1, 4, 9, 12, 13]. This situation can be prone due to more trauma with age and footwear. The frequency according to gender is controversial [12–14]. In the present study, consistent with the data, [1, 4, 9, 12, 13] mold onychomycosis was more frequent in toe-nails than in finger-nails and over age 40. Of the patients 76% were females.

Different molds such as *Scopulariopsis brevicaulis; Fusarium, Aspergillus, Acremonium, Curvularia, Chrysosporium, Penicillium, Alternaria* spp. and some others are reported as causative agents of onychomycosis [1, 3, 7, 9–11, 14]. In Spain [7] and Italy [9] *S. brevicaulis* is the dominant causative mold with 7.8 and 35%, respectively. In Estonia *Acremonium* and *Scopulariopsis* spp. have been the most frequent molds isolated in toe-nail onychomycosis [11]. In America *Fusarium* spp. seem to be the most frequent agents [1, 10] attaining a frequency of 34.1% in the United States [10]. In India *Aspergillus* spp. have the lead with 86.4% [3]. A recent paper from Spain [8] reports the increasing prevalence of *Aspergillus versicolor* onychomycosis. Also in this study, like in India [3], *Aspergillus* spp. are found to be the predominating causative molds (32%), *A. niger* showing a prevalence of 22% (Table 2). In contrast to our finding, in a previous study in Turkey [14], *S. brevicaulis* was reported to be the most frequent mold with a frequency of 1.9%. Also rare molds such as *Hendersonula toruloidea* [15, 16], *Exophiala jeanselmei* [17], and some others are reported as causative agents. Data confirm a large scope of molds as agents of onychomycosis with therapeutic implications due to their possible drug resistance [6, 18–21].

As shown in the present study, the definite diagnosis of a ''mold'' onychomycosis is not as easy as the diagnosis of onychomycoses due to only dermatophytes and yeasts. In view of the therapeutic implications of ungual infections due to molds, laboratory staff should consider also molds as agents in cases clinically prediagnosed as onychomycosis, but not yielding dermatophyte or yeast growth in culture. They should set up consecutive cultures from consecutive nail scrapings to reach dependable laboratory results.

References

1. Velez H, Diaz F. Onychomycosis due to saprophytic fungi. Report of 25 cases. Mycopathologia 1985; 91: 87–92.
2. Lim JT, Chua HC, Goh CL. Dermatophyte and non-dermatophyte onychomycosis in Singapore. Australas J Dermatol 1992; 33: 159–163.
3. Ramani R, Srinivas CR, Ramani A, Kumari TG, Shivananda PG. Molds in onychomycosis. Int J Dermatol 1993; 32: 877–878.
4. Ginter G, Rieger E, Heigl K, Propst E. Increasing frequency of onychomycoses – is there a change in the spectrum of infectious agents?. Mycoses 1996; 39(Suppl. 1): 118–122.
5. Ellis DH, Watson AB, Marley JE, Williams TG. Non-dermatophytes in onychomycosis of the toenails. Br J Dermatol 1997; 136: 490–493.

6. Gupta AK, Jain HC, Lynde CW, Watteel GN, Sumerbell RC. Prevalence and epidemiolgy of unsuspected onychomycosis in patients visiting dermatologists' offices in Ontario, Canada – a multicenter survey of 2001 patients. Int J Dermatol 1997; 36: 783–787.
7. Vélez A, Linares MaJ, Fenández-Roldán JC, Casal M. Study of onychomycosis in Córdoba, Spain: prevailing fungi and pattern of infection. Mycopathologia 1997; 137: 1–8.
8. Torres-Rodriguez JM, Madrenys-Brunet N, Siddat M, Lopez-Jodra O, Jimenez T. *Aspergillus versicolor* as a cause of onychomycosis: report of 12 cases and susceptibility testing to antifungal drugs. J Eur Acad Dermatol Venereol 1998; 11: 25–31.
9. Gianni C, Gerri A, Crosti C. Non-dermatophytic onychomycosis. An underestimated entity? A study of 51 cases. Mycoses 2000; 43: 29–33.
10. Ghannoum MA, Hajjeh RA, Scher R. 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 2000; 43: 641–648.
11. Järv H, Naaber P, Kaur S, Eisen M, Silm H. Toe-nail onychomycosis in Estonia. Mycoses 2003; 47: 57–61.
12. Gupta AK, Gregurek-Novak T, Konnikov N, Lynde CW, Hofstader S, Summerbell RC. Itraconazole and terbinafine treatment of some non-dermatopytic molds causing onychomycosis of the toes and a review of the literature. J Cutan Med Surg 2001; 5: 206–210.
13. Canteros GE, Davel GO, Vivot W. Causal agents of onychomycosis [Abstract]. Rev Argent Microbiol 1994; 26: 65–71.
14. Kiraz M, Yeğenoğlu Y, Erturan Z, Anğ Ö. The epidemiology of onychomycosis in Istanbul, Turkey. Mycoses 1999; 42: 323–329.
15. Liony C, Joly P, Balguerie X, Fusade T, Lauret P. Cutaneous and nail infections caused by *Hendersonula toruloidea*. Ann Dermatol Venereol 1993; 120: 226 228.
16. Elewski BE. Onychomycosis caused by *Scytalidium dimidiatum*. J Am Acad Dermatol 1996; 35: 336–338.
17. Boisseau-Garsaud AM, Desbois N, Guillermin ML, Ossondo M, Gueho E, Cales-Quist D. Onychomycosis due to *Exophiala jeanselmei*. Dermatology 2002; 204: 150–152.
18. Nolting S, Brautigam M, Weidinger G. Terbinafine in onychomycosis with involvement by non-dermatophytic fungi. Br J Dermatol 1994; 130(Suppl. 43): 16–21.
19. Tosti A, Piraccini BM, Stinchi C, Lorenzi S. Onychomycosis due to *Scopulariopsis brevicaulis*: clinical features and response to systemic antifungals. Br J Dermatol 1996; 135: 799–802.
20. Baykal C, Kazancioğlu R, Büyükbabani N. Simultaneous cutaneous and ungual alternariosis in a renal transplant recipient. Br J Dermatol 2000; 143: 910–912.
21. Llovo J, Prieto E, Vazquez H, Muñoz A. Onychomycosis due to *Onychocola canadensis*: report of the first two Spanish cases. Med Mycol 2002; 40: 209–212.

Address for correspondence: Dr. S. Hilmioğlu-Polat, MD, Departments of Microbiology & Clinical Microbiology, Mycology Laboratory, Faculty of Medicine, Ege University, TR-35100 Bornova, Izmir, Turkey
Phone: +90-232-390-33-033; Fax: +90-232-3422142
E-mail: hilmiog@med.ege.edu.tr