Onychomycosis in North-East of Iran

Parvaneh Afshari, Sadegh Khodavaisy, Shamsi Kalhori, Maryam Ghasemi, Taraneh Razavyoon

1Medical Mycology Laboratory of Referral Laboratory, Mazandaran University of Medical Sciences, Sari, Iran. 2Department of Medical Mycology and Parasitology, Tehran University of Medical Sciences, Tehran, Iran. 3Department of Medical Mycology and Parasitology, Kurdistan University of Medical Sciences, Sanandaj, Iran. 4Department of Pathology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. 5Jahan Clinical Laboratory, Tehran, Iran.

Received: January 2014, Accepted: March 2014.

ABSTRACT

Background and Objectives: Onychomycosis is a common fungal infection which has been conducted in many parts of the world. The aim of this study was to evaluate the epidemiology and to identify the aetiological factors of onychomycosis in Mazandaran province, Iran.

Materials and Methods: During the period of 10 years (2003–2012) 1100 patients suspected with onychomycosis, referred to the Mycology Laboratory of the Referral Laboratory and Boali Sina Hospital of Mazanadaran University of Medical Sciences, Sari, Iran, were assessed for the presence of onychomycosis with mycological examination based on conventional techniques.

Results: Among 1100 subjects (398 males and 702 females, aged 1-88 years) onychomycosis was diagnosed in 625 (56.8%) cases. Among cases of onychomycosis, laboratorial confirmation was reached through direct examination with positive cultures in 464 samples (74.3%), while only by positive direct exam in 114 cases (18.2 %) or just positive culture in 47 cases (7.5%). The results of fungal culture revealed Candida spp. isolated in (61.9%) of the cases as the most common agents of onychomycosis while among dermatophytes, Trichophyton mentagrophytes was found in 17.7% followed by T. rubrum (1.7%), Epidermophyton floccosum (0.7%), T. violaceum (0.2%), T. verrucosum (0.2%), T. tonsurans (0.2%) and Microsporum gypseum (0.2%). Among the non-dermatophyte moulds, Aspergillus spp. was the most prevalent species (14.2%).

Conclusion: The results demonstrated that onychomycosis was diagnosed in 625 (56.8%) cases and the most common isolates were Candida spp., followed by dermatophytes and moulds. This epidemiological data collected may be useful in the development of preventive and educational strategies.

Keywords: Onychomycosis, Dermatophytes, Nondermatophyte, Candida, Iran

INTRODUCTION

Onychomycosis is a common fungal infection affecting both fingernails and toenails which usually caused by dermatophytes, yeasts and molds (1). Onychomycosis is classified into five clinical types, according to the fungal invasion of the nail: distal and/or lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), proximal subungual onychomycosis (PSO), total dystrophic onychomycosis (TDO), and Candida onychomycosis (CO) (1, 2). This disease is still considered as major public health problem in many parts of the world. The prevalence of onychomycosis varies in different geographical areas and with the passing of the time depending on several factors, especially different environmental and lifestyle conditions (3–6). Several
epidemiological studies evaluated in different countries indicate that onychomycoses represent up to 50% of all nail disorders and 30% of all superficial mycoses (7). Because of increase in the prevalence of onychomycosis during the last decades, investigations on causative fungal agents and determination the role of various types of climate, socio-economical, occupational situations, in the epidemiology of this disease is necessary (8). Like other parts of the world, onychomycosis is also a common clinical presentation in Iran. Nevertheless, little information exists on the prevalence of the pathogens and their clinical presentation in some provinces of Iran (9-12). The aim of the present retrospective study which was performed for evaluation of the etiological and epidemiological factors of the onychomycosis in Sari, Mazandaran province, Iran in a 10 years period.

MATERIALS AND METHODS

Study population. During a period of 10 years (2003-2012), 1100 patients clinically suspected to onychomycosis, referred to the Mycology Laboratory of the Referral Laboratory and Boali Sina Hospital of the Mazanadaran University of Medical Sciences, Iran were recruited. 63.8% of patients were females. The average age of patients was 37 years (ranging from 1 to 88 years).

Sample collection. For all patients in this study, a questionnaire was completed that contained demographic data, patient history and specific data related to predisposing factors for onychomycosis. The technique used to collect specimens depends on the site of the infection. In distal subungual onychomycosis, the nail clipped short, and a small curette or scalpel blade used to obtain a specimen from the nail bed as close to the cuticle as possible. A specimen was also taken from the underside of the nail plate. In white superficial onychomycosis, a blade or curette used to scrape the nail surface or the white area, and remove infected debris. In proximal superficial onychomycosis, the healthy nail plate gently pared away with a scalpel blade. A sharp curette used to remove material from the infected proximal nail bed as close to the lunula as possible. In candidal onychomycosis, infected material collected from the proximal and lateral nail edges.

Mycology investigation. A diagnosis was established when clinical manifestations were combined with a positive direct microscopic examination and/or culture documented a pathogenic species. Nail material was digested in KOH/DMSO (20% potassium hydroxide solution mixed in 40% dimethyl sulphoxide V/V) directly on a microscope slide and tested with Bright field microscope for the presence of fungal elements. In the negative samples, the utilization of a KOH/CFW (0.1% aqueous solution of calcofluor white mixed in equal volumes with the potassium hydroxide) can allow for earlier recognition of the fungus in tissue under ultra violet illumination.

For primary isolation, Nail material samples were cultivated in tubes containing Sabouraud dextrose agar (SDA; Merck, Darmstadt, Germany) with and without 0.05% cyclohexamide and 0.005% chloramphenicol for pathogenic fungi. The tubes were incubated at 25-30°C for up to 4 weeks and examined at 2 to 3 day intervals for fungal growth. Macroscopic and microscopic characteristics were analyzed for genus and species identification. Identification of the etiological agents performed based on the gross morphology of the fungal colony (texture, color, surface and revers pigment, topography) and microscopic characterization of their hyphae and conidia (type of macroconidia, shape and size of micro conidia). In some cases, they sub cultured on Potato dextrose agar (PDA; Merck, Darmstadt, Germany) or slide cultures and other biochemical tests used final identify.

RESULTS

Among 1100 clinically suspected cases of onychomycosis, 625 patients (56.8%) were confirmed to be affected with onychomycosis. The study population comprised 393 (62.9%) females and 232 (37.1%) males, ranging in age from 1 to 88 years (mean age; 37 years). The individuals most frequently involved were aged between 30-39 years 152 people (24.3%) then between 20-29 years 128 (20.5%) and 40-49 years 118 (18.9%) respectively (Table 1). The results indicate that laboratorial confirmation was reached through direct examination with positive cultures in 464 samples (74.3%), while only by positive direct exam in 114 cases (18.2%) or just positive culture in 47 cases (7.5%). Among cases of onychomycosis, direct examination and culture were positive for yeast in 330 (52.8%) individuals; for filamentous in 276 (44.2%) individuals; for mixed yeast
and filamentous in 19 (3%) individuals. Fingernail onychomycosis was recognized in 356 (57%), toenail onychomycosis in 252 (40.3%) and both in 17 (2.7%) cases. The most commonly isolated dermatophyte was *Trichophyton mentagrophytes* 94(17.7%), followed by *T. rubrum* 9(1.7%), *Epidermophyton floccosum* 4(0.7%), *T. violaceum* 1(0.2%), *T. verrucosum* 1(0.2%), *T. tonsurans* 1(0.2%) and *Microsporum gypseum* 1(0.2%) while the most isolated yeast was *Candida* spp. 328 (61.9%). Among the nondermatophyte moulds, *Aspergillus* spp. was the most prevalent species 75(14.2%) followed by, *Fusarium* spp. 2(0.4%), *Scopulariopsis* spp. 7(1.3%) and *Penicillium* spp. 4(0.7%), *Cladosporium* spp.

| Age group | Total | >70 | 60-69 | 50-59 | 40-49 | 30-39 | 20-29 | 10-19 | 0-9 |
|-----------|-------|-----|-------|-------|-------|-------|-------|-------|-----|
| Male      | 2     | 2   | 7     | 14    | 12    | 9     | 7     | 8     | 13  |
| Female    | 5     | 3   | 12    | 23    | 18    | 14    | 10    | 14    | 15  |
| Total     | 7     | 5   | 19    | 37    | 30    | 26    | 17    | 22    | 28  |

Table 1. Prevalence of agents isolated from onychomycosis according to the age and sex.

| Agents                        | Finger                  | Toe                     | Both                     | Total |
|-------------------------------|-------------------------|-------------------------|--------------------------|-------|
| *Trichophyton mentagrophytes* | 18 (21.4)               | 64 (76.2)               | 2 (2.4)                  | 84    |
| *Trichophyton rubrum*         | 2 (22.2)                | 7 (77.8)                | -                        | 9     |
| *Epidermophyton floccosum*    | -                       | 3 (75)                  | 1 (25)                   | 4     |
| *Trichophyton violaceum*      | -                       | 1 (100)                 | -                        | 1     |
| *Trichophyton verrucosum*     | -                       | -                       | 1 (100)                  | 1     |
| *Trichophyton tonsurans*      | 1 (100)                 | -                       | -                        | 1     |
| *Microsporum gypseum*         | -                       | 1 (100)                 | -                        | 1     |
| Mixed *Trichophyton mentagrophytes* &*Candida* spp. | 7 (70) | 1 (10) | 2 (20) | 10 |
| *Candida* spp.                | 258 (83.5)              | 45 (14.5)               | 6 (2)                    | 309   |
| Mixed *Aspergillus flavus* & *Candida* spp. | 5 (55.5) | 4 (44.5) | - | 9 |
| *Aspergillus fumigatus*       | 1 (25)                  | 3 (75)                  | -                        | 4     |
| *Aspergillus flavus*          | 13 (31.7)               | 27 (65.9)               | 1 (2.4)                  | 41    |
| *Aspergillus* spp.            | 6 (28.6)                | 15 (71.4)               | -                        | 21    |
| *Fusarium* spp.               | -                       | 2 (100)                 | -                        | 2     |
| *Scopulariopsis* spp.         | 2 (28.6)                | 5 (71.4)                | -                        | 7     |
| *Geotrichum* spp.             | 1 (100)                 | -                       | -                        | 1     |
| *Trichosporon* spp.           | -                       | 1 (100)                 | -                        | 1     |
| *Cladosporium* spp.           | -                       | 1 (100)                 | -                        | 1     |
| *Penicillium* spp.            | 1 (25)                  | 3 (75)                  | -                        | 4     |
| Culture negative              | 41 (36)                 | 69 (60.5)               | 4 (3.5)                  | 114   |
| Total                         | 356 (57)                | 252 (40.3)              | 17 (2.7)                 | 625   |

Table 2. Distribution of patients with onychomycosis according to site of infection.
ONYCHOMYCOSIS IN NORTH-EAST OF IRAN

101

http://ijm.tums.ac.ir

IRAN. J. MICROBIOL. Vol. 6, No. 2 (April 2014), 98-103

1(0.2%), Geotrichum spp. 1(0.2%) and Trichosporon spp. 1 (0.2%) (Table 2).

DISCUSSION

The results demonstrated that Onychomycosis is a chronic infection of the toe and finger nails; that is a growing public health concern all over the world (9). Our data revealed that onychomycosis was proved in 625 (56.8%) cases which is almost similar to the frequencies reported from Isfahan and Qazvin provinces of Iran (10, 11), but is higher than reported from Tehran (2.4%) (12). The prevalence of onychomycosis in Europe ranges from 2 to 8% depending on the country (13-15). The East Asian study showed the prevalence of onychomycosis was 16.6% in Hong Kong (16). Two separate studies conducted in Turkey demonstrated the prevalence of onychomycosis as 0.1% (17, 18). A higher prevalence of onychomycosis (85%) was reported in the Muslim community of Durban, South Africa (19). In this study the ratio of male (37.1%) to female (62.9%) onychomycosis patients was approximately 1:3 that similar frequency has been reported in other studies, higher incidence of onychomycosis in women than men (15, 20-24). Because most of the women in the perusal were housewife and prolonged moisture and cosmetic reasons may account for this. This survey noticed the prevalence of onychomycosis increased gradually with the age of the patients. The most cases found in middle aged people (the tertiary to quintuplicate). Similar observations were reported with other studies (11, 22, 24, 26, 27), that is because of having more social activities and more exposure to fungal elements. This study supported that onychomycosis affected mainly fingernails in compared to toenails. In another study, in Isfahan, 141 (72.7%) cases with fingernail onychomycosis and 53 (27.3%) cases with toenail onychomycosis were reported (10). Toenails are affected more often than fingernails, probably due to their slow growth, which facilitates invasion of the aetiological agent and is perhaps supported by events such as traumas and poor circulation (28). The prevalence of onychomycosis due to dermatophytic origin found in this survey was higher in toenails (71%) than in fingernails (29%). Furthermore, fungal infection of toenails with dermatophytes, the most common isolated agent, was non dermatophytes molds and then yeasts. Yeasts can cause onychomycosis but mixed infections with dermatophytes are also possible. The genus Candida was the most common species detected in this study with an isolation rate of 61.9% (n = 328). High rate of isolation for this genus, with a rate of almost 50%, had also been reported from Isfahan nd Qazvin provinces (10, 11) which is similar to findings in other studies (10-20, 29-31). In contrast to our observation, in a US epidemiological survey it was noted that yeast and non-dermatophytic moulds play a minor role in onychomycosis (32) which is different from other reports that found dermatophytes as the predominant aetiological agent (33-35). Such differences may be related to local environmental conditions.

In our study Trichophyton mentagrophytes was the most common dermatophytes isolated. Similar finding was reported from from Iran and other countries (1, 10, 11, 22, 36). A significant increase in the prevalence of T. mentagrophytes and T. rubrum over the last decades may perhaps be due to the greater availability of fungi in the environment and in adults after prolongation of life accompanied by various diseases (37, 38). Our study also demonstrated that commonness of onychomycosis due nondermatophytic agents was higher in toenails (66%) than in fingernails (34%) and Aspergillus spp. Aspergillus flavus was particularly the predominant isolated organism, followed by scopulariopsis spp. from toenails in both sex.

In conclusion, this study showed that onychomyco -sis in population study was proved in 56.8% cases. The most common agents were Candida spp., followed by dermatophytes and other filamentous fungi. This survey may be useful in the development of preventive and educational strategies, and consequently in reducing healthcare expenditure. Physicians should consider this infection in the differential diagnosis of diseases affecting nails and provide valuable epidemiological data on future efforts for the prevention and treatment of onychomycosis. Also, epidemiological investigations should be performed in every area in order to determine the fungal species associated with onychomycosis.

ACKNOWLEDGEMENTS

This study was supported by Research Deputy of the Mazandaran University of Medical Sciences (Project HSR 91-4). The authors are grateful to Dr. Nima Motamed for his help in part of the statistical analysis of the manuscript. We wish to thank the staff of Boali Sina Hospital and the
Referral Laboratory of the Mazandaran University of Medical Sciences.

REFERENCES

1. Faergemann J, Baran R. Epidemiology, clinical presentation and diagnosis of onychomycosis. Br J Dermatol 2003; 149: 1-4.
2. Nannedkar-Thomas MA, Escher RK. An update on disorders of the nails. J Am Acad Dermatol 2005; 52: 877-877.
3. Perea S, Ramos MJ, Garau M, Gonzalez A, Noriega AR, del Palacio A. Prevalence and risk factors of tinea unguium and tinea pedis in the general population in Spain. J Clin Microbiol 2000; 38: 3226-3230.
4. Hanke E, Roseeuw D. The scope of onychomycosis: epidemiology and clinical features. Int J Dermatol 1999; 38(Suppl. 2): 7-12.
5. Sigurgeisson B, Steingrimsson O. Risk factors associated with onychomycosis. J Eur Acad Dermatol Venereol 2004; 18: 48-51.
6. Cheng S, Chong L. A prospective epidemiological study on tinea pedis and onychomycosis in Hong Kong. Chinese Medical Journal 2002; 115: 860-865.
7. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. Mycopathologia 2008; 166: 335-352.
8. Gerami Shoar M, Zomorodian K, Emami M, Tarazoei B, Saadat F. Study and identification of the etiological agents of onychomycosis in Tehran, capital of Iran. Iran J Publ Health 2002; 31: 100-104.
9. Shokohi T, Hajheidari Z, Haghani A, Khaliilian A, Ahgili SR, Miah S. The study of 101 cases of onychomycosis and associate factors in patients referred to Boali Sina Hospital and Toba dermatology outpatient clinics in Sari. J Mazandaran Uni Med Sci 2009; 19: 33-43.
10. Chadeganpour M, Nilipour S, Ahmadi Gh. Study of onychomycosis in Isfahan, Iran. Mycoses 2008; 53: 153-157.
11. Aghamirinia M R, Ghiasian S A. onychomycosis in Iran; epidemiological and causative agent and clinical features. Jpn J Med Mycol 2010; 51: 23-29.
12. Falahati M, Akhlaghi L, Lari AR, Alaghehbandan R. Epidemiology of dermatophytes in an area south of Tehran, Iran. Mycopathologia 2003; 156: 279-287.
13. Svejgaard EL, Nilsson J. Onychomycosis in Denmark: prevalence of fungal nail infection in general practice. Mycoses 2004; 47: 131-135.
14. Pereiro M Jr, Toribio J. Epidemiology de onychomycose. Journal de Mycologie Medicale 2002; 12: 175-182.
15. Sais G, Gueala A, Peyry J. Prevalence of dermatophytes onychomycosis in Spain: a cross sectional study. Br J Dermatol 1995; 132: 758-761.
16. Cheng S, Chong L. A prospective epidemiological study on tinea pedis and onychomycosis in Hong Kong. Chinese Med J 2002; 115: 860-865.
17. Gunduz T, Metin DY, Sacar T, Hiliomioglu S, Baydur H, Inci R, et al. Onychomycosis in primary school children: Association with socioeconomic conditions. Mycoses 2006; 49: 431-433.
18. Metintas S, Kiraz N, Arslantas D, Akgun Y, Kalyoncu C, Kiremitci A, et al. Frequency and risk factors of dermatophytosis in students living in rural areas in Eskiehir, Turkey. Mycopathologia 2004; 157: 379-382.
19. Raboobee N, Abboobaker J, Peer AK. Tinea pedis et unguium in the Muslim community of Durban, South Africa. International Journal of Dermatology 1998; 37: 759-765.
20. Alvarez MI, Gonzalez LA, Castro LA: Onychomycosis in Cali, Colombia. Mycopathologia 2004; 158: 181-186.
21. Mercantini R, Marsella R, Moreto D. Onychomycosis in Rome, Italy. Mycopathologia 1996; 136: 25-32.
22. Ravinder Kaur, Bineeta Kashyap, Preena Bhalla. A five-year survey of onychomycosis in new delhi, india: epidemiological and laboratory aspects. Indian J Dermatol 2007; 52: 39-42.
23. Aman S, Haroon TS, Hussain I, Bokhari MA, Khurshid K. Tinea unguium in Lahore, Pakistan. Med Mycol 2001; 39: 177-180.
24. Banerjee U, Sethi M, Pasiucha JS. Study of onychomycosis in India. Mycoses 1990; 33: 411-415.
25. Gupta AK, Jain HC, Lynde CW, Watteel GN, Summerbell RC. Prevalence and epidemiology of unsuspected onychomycosis in patients visiting dermatologists’ offices in Ontario, Canada a multicenter survey of 2100 patients. Int J Dermatol 1997; 36: 783-787.
26. Jorge O, Lopes, Sydney H. Alves, Cristine RD, Mari, Loiva TO, et al. A ten-year survey of onychomycosis in the central region of the rio grande do sul, Brazil. Rev Inst Med trop. S. Paulo 1999; 41: 147-149.
27. Khosravi AR, Aghamirian MR, Mahmoudi M: Dermatophytes in Iran. Mycoses 1994; 37: 43-8.
28. Hay R. Literature review: Onychomycosis. J Eur Acad Dermatol Venereol 2005; 19 (Suppl. 1): 1-7.
29. Nsanze H, Lestringant GG, Mustafa N, Usmani MA. Aetiology of onychomycosis in Al Ain, United Arab Emirates. Mycoses 1995; 38: 421-424.
30. Pontes ZB, Lima EdeO, Oliveira NM, Dos Santos JP, Ramos AL, Carvalho MF. Onychomycosis in Joao Pessoa city, Brazil. Rev Argent Microbiol 2002; 34: 95-99.
31. Romano C, Gianni C, Difonzo EM. Retrospective study of onychomycosis in Italy: 1985-2000. Mycoses 2005; 48: 42-44.
32. Aman S, Haroon TS, Hussain I, Bokhari MA, Khurshid K. Tinea unguium in Lahore, Pakistan. Med Mycol 2001; 39: 177-180.
33. Khosravi AR, Mansouri P. Onychomycosis in Tehran, Iran: prevailing fungi and treatment withitraconazole. Mycopathologia 2000; 150: 9-13.
34. Ng KP, Saw TL, Madasamy M, Soo Hoo TS. Onychomycosis in Malaysia. Mycopathologia 1999; 147: 29-32.
35. Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: prevailing fungi and pattern of infection. Indian J Med Microbiol 2007; 25: 53-56.
36. El sayed F, Ammoury A, Haybe RF, Dhaybi R.
Onychomycosis in Lebanon: a mycological survey of 772 patients. *Mycoses* 2006; 49: 216-219.

37. Tsoumani M, Jelastopulu E, Bartzavali C, Vamvakopoulou S, Dimitracopoulos G, Anastassiou ED, et al. Changes of dermatophytoses in southwestern Greece: An 18-year survey. *Mycopathologia* 2011; 172: 63-67.

38. Leibovici V, Evron R, Dunchin M, Westerman M, Ingber A. A population-based study of toenail onychomycosis in Israeli children. *Pediatr Dermatol* 2009; 26: 95-97.