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Onychomycosis in North-East of Iran

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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

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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

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

		Age group								
	sex	0-9	10-19	20-29	30-39	40-49	50-59	60-69	>70	Total
Yeast	Male	13	9	12	22	14	12	8	7	97
	Female	15	30	45	63	40	16	14	10	233
	Total	28	39	57	85	54	28	22	17	330
Filamentous fungi	Male	2	8	24	26	26	17	17	9	129
	Female	2	10	42	37	32	13	7	4	147
	Total	4	18	66	63	58	30	24	13	276
Mixed	Male	-	-	1	2	1	1	1	-	6
	Female	-	1	4	2	5	-	1	-	13
	Total	-	1	5	4	6	1	2	-	19
Total		32	58	128	152	118	59	48	30	625

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. tonsourans* 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.

Table 2. Distribution of patients with onychomycosis according to site of infection.

Agents	Clinically Variation			Total
	Finger N (%)	Toe N (%)	Both N (%)	
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 tonsourans	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
Scopolariopsis 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

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

DISCUSSION

The results demonstrated that Onychomychosis 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 prodominant 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.

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