



Tinea unguium in the North-West of Iran (1996-2004)

Abdolhassan Kazemi

National Public Health Management Center (NPMC), Infectious and Tropical Diseases Research Center & Biotechnology Research Center (BRC), Tabriz University of Medical Sciences Tabriz, Iran

Summary *Tinea unguium* is a common mycosis in many part of the world including Iran. The prevalence of this mycosis varied depending on time, health level and geographical location. To stabilise the etiological, epidemiological and risk factors of *tinea unguium* in North-west Iran, a study of patients with suspected dermatophyte infections of their nails was carried out between 1996 and 2004. During this study 590 (354 females and 236 males) patients with clinical presentation of fungal infection in fingernails, toenails or in the both sites, were investigated using direct microscopy and culture of clinical samples. *Tinea unguium* was documented in 41 cases (7%) and among positive cases, 16 cases (39% total positive cases) were female and 25 cases (61% total positive cases) were male. Seventeen patients (41% total positive cases) had *tinea unguium* in their finger nails and 24 patients (59% total positive cases) had infection in their toe nails. According to the isolated etiologic agent, 66% (19 cases) of *tinea unguium* infections were caused by zoophilic dermatophytes, 31% (9 cases) were caused by anthropophilic dermatophytes and 3% (1 case) were caused by geophilic dermatophytes. With regard of sex, *tinea unguium* did not show a significant difference. The highest prevalence of *tinea unguium* was found in patients between 11 and 40 years of age. In conclusion the current results identified the etiological agents and epidemiological aspects of *tinea unguium* in North-west Iran. *Tinea unguium* in this region is associated with animal husbandry and direct or indirect contact with their products (wool, leather)

Key words *Tinea unguium*, Dermatophytes, Iran

Tinea unguium en el noroeste de Irán (1996-2004)

Resumen *Tinea unguium* es una micosis común en muchas partes del mundo, incluyendo Irán. La incidencia de esta micosis puede variar dependiendo del tiempo, salud y localidad geográfica. Para averiguar la etiología, epidemiología y factores de riesgo de *tinea unguium*, se estudiaron pacientes con uñas con posible de infección por dermatofitos entre 1996-2004. Durante este estudio, 590 pacientes (354 mujeres y 236 varones) con clínica probable de infección fúngica en las uñas de manos, pies o en ambos, fueron investigados mediante examen directo, así como por cultivos de muestras clínicas. *Tinea unguium* se presentó en 41 casos (7%) y, de entre los casos positivos, 18 (44% del total de casos positivos) fueron mujeres y 23 (56% del total de casos positivos) fueron varones. Diecisiete pacientes (41% del total de casos positivos) presentaban *tinea unguium* en uñas de las manos y 24 (59% del total de casos positivos) presentaban infección en las uñas de los pies. Desde un punto de vista etiológico, en el 65,5% (19 casos) las onicomicosis fueron causadas por dermatofitos zoofilos, en el 31% (9 casos) por antropófilos y en el 3,5% (1 caso) por geofilos. *Tinea unguium* no presentó diferencias significativas entre sexos. La mayor presencia se dio en pacientes entre 11-40 años de edad. En definitiva, parece que en el noroeste de Irán *tinea unguium* está asociada con el cuidado de animales y el contacto directo o indirecto con sus productos (lana, cuero).

Palabras clave *Tinea unguium*, Dermatofitos, Irán

Corresponding author:

Abdolhassan Kazemi
National Public Health Management Center (NPMC),
Infectious and Tropical Diseases Research Center &
Biotechnology Research Center (BRC)
Tabriz University of Medical Sciences
Tabriz, Iran
Tel.: (+ 98) (0)411 6578978
Fax: (+ 98) (0)411 6578974
E-mail: Hassan5628@yahoo.com

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The nails of the hands and feet can be infected by various types of dermatophyte such as: zoophilic, anthropophilic and geophilic dermatophyte [39]. Such infections may occur by different means and involve different parts of the nails. There are striking geographical differences in the epidemiological and etiological pattern of *tinea unguium*, especially in the frequency with which each group of dermatophyte is responsible for infection [25]. In general, fungal diseases of nail demonstrate major differences in different regions of the world: for example, dermatophytes are the main etiological agents of onychomycosis in countries such as India and UK [14], Germany [25], the USA [13], Spain [32,38] and Canada [25], whilst a high prevalence of candidal onychomycosis has been reported in Cordoba [43], Belgium [24] and Saudi Arabia [2]. Moreover, in recent years, other non-dermatophytic filamentous fungi, such as *Scytalidium* species, have been frequently reported as the etiological agent of onychomycosis from tropical regions [28]. Other filamentous saprophytic fungi, such as *Acremonium*, *Aspergillus*, *Fusarium*, *Scopulariopsis*, *Penicillium*, *Hendersonula*, *Onychocola* and *Ulocladium* species, have also been recorded as agents of onychomycosis [3,10,11,15-18,40,41]. The changes of life style related to new customs also appear to play an important role in fungal nail infection. For instance, a report has shown an association with sport activities and the type of shoes worn [3]. Our study suggested a probable association between animal husbandry, long term boot and high boot wearing, direct and indirect contact with either animals or wool, leather and *tinea unguium* in North-west area of Iran. It is necessary to pay attention to the fact that, in this area of Iran the keeping of sheep and cattle is one of the main and public economic activities.

With regard to appropriate diagnosis *tinea unguium* infection present some problems since dermatophytic nail infection may be mistaken for other onychomycoses or other nail diseases (e.g. psoriasis, onycholysis, etc). This matter is extremely important from a therapeutic point of view. Accurate analysis should be carried out not only to be certain that fungus is present in infected nail, but also to identify the dermatophyte by: 1) Direct microscopic examination, 2) Culture of clinical samples, 3) Molecular methods, such as PCR [44].

Materials and methods

Five hundred ninety patients, showing nail lesions of probable fungal origin, were included in this study. Patients were from various regions of North-west Iran. The age group of patients were from 1 to 70 years of age. After clinical examination by a dermatologist, suspected cases with brittle, friable, thickened or cracked nails, yellow to brown nails, nails with whitish spots or nails with an opaque, circumscribed area on the surface of the nail plate were sent for para-clinical examination. Any other *tinea unguium* forms of nail dystrophy were also included.

Fungal material was collected from all of the patients after careful cleaning of nails with 70% ethanol. Nail scrapings and clippings were collected from both the lower and upper layer of the infected nail and as close as possible to the healthy part of the nail. A sterile scalpel or lancet was used. The samples were placed on sterile Petri dishes and divided into two parts (for culture and direct microscopic examination).

Direct microscopic examination of the nail specimens was carried out in 20% potassium hydroxide solution on a sterile glass slide. The slides were heated gently over a low flame and examined using direct light microscopy.

The morphology and amount of hyaline branched, septate hyphae were recorded, along with the presence of single or chains of arthroconidia. All results were reported to the dermatologist on the same day.

The second half of the sample was inoculated onto various media in duplicate, including Dermatophyte Test Medium (DTM), Sabouraud's dextrose agar (SDA) with 0.05 mg/ml Chloramphenicol (SC) and SDA with 0.5 mg/ml Cycloheximide and 0.05 mg/ml Chloramphenicol (SCC) in tubes. The tubes were incubated at both 20-25 °C (RT) and 37 °C for four weeks. Lactophenol Cotton Blue (LCB) preparations were made from positive cultures and examined under the microscope. Identification of the isolates also included macromorphological examination of the colonies, culture characters, pigment production on the surface and under the surface of the colonies, hair perforation and urease production in urea media and slide-culture preparation. Colonies without micro and macroconidia were re-cultured on rice grain media. When direct microscopic examination was positive and cultures were negative, the mycological investigation was repeated again in at least two additional occasions.

Results

The collected data were examined in various ways. Firstly, it should be noted that some clinical samples were positive by direct microscopic examination, but negative on culture. This is an acceptable result in the laboratory diagnosis of *tinea unguium*. In presenting the results, aspects that allow correlating of the pathology of *tinea unguium* with etiological, sex, age, risk factors and other epidemiological aspects, are emphasised.

Five hundred ninety patients with suspected infections of their nails were included in this study [354 females (60%) and 236 males (40%)]. The age of patients were 1-70 years but there were only 1 male (2%) patients in age group 61-70 and 29 patients (71%) [11 females (27%), 18 males (44%)] in age group 11-40 therefore, the highest prevalence of mycosis was found in cases between 11-40 years of age with a significant differentiation between females and males (16/354 vs. 25/236; $p < 0.05$). Of the 590 subjects, dermatophyte elements were present by direct microscopic examination in 41 cases (7%) of which 16 cases (4.5%) total number female and 44% total positive cases) were female, therefore the number of positive males were 25 cases (10.5%) total suspicious males and 55% total positive cases). The rates of negative cases were 93% (549 cases) of total suspicious subjects. Therefore, this study deals with the positive results observed in 4.5% (16 cases) total females and 10.5% (25 cases) total males while, suspicious females were 354 cases but suspicious males were 236 cases. Consequently, data showed a significant difference between the number of females and males with a positive result ($p < 0.05$). In case of consideration positive result in both direct examination and culture of clinical sample, only 29 (4.9%) of patients were positive. There are some reasons for negative culture result from samples which were positive in direct microscopy. These reasons have been mentioned in discussion.

Of the 41 patients with *tinea unguium*, 17 (41%) initially had finger nails infections [7 female (17%) and 10 males (24%)]. Twenty-four patients (59%) initially had this infection in their toe nails [9 female (22%) and 15 male (37%)]. In some patients with chronic involvement, infection was seen in both hands and feet nails. The highest prevalence of *tinea unguium* was seen in cases between 11-40 years of age. Males were infected more fre-

Table 1. Number of finger and toe nail cases of *tinea unguium*.

	Finger nails		Toe nails		Total
	Male (%)	Female (%)	Male (%)	Female (%)	
Positive	10 (24%)	7 (17%)	15 (37%)	9 (22%)	41
Negative	118 (21.5%)	177 (32%)	93 (17%)	161 (29.5%)	549
Total	128 (22%)	184 (31%)	108 (18%)	170 (29%)	590

Table 2. Number of positive cases in relation to dermatophyte group and species

Group	Dermatophyte	Finger nails	Toe nails	Total (%)
Zoophilic (66%)	<i>T. verrucosum</i>	3	3	6 (21%)
	<i>T. mentagrophytes</i>	4	5	9 (31%)
	<i>M. canis</i>	1	3	4 (14%)
Anthropophilic (31%)	<i>T. rubrum</i>	1	1	2 (7%)
	<i>E. floccosum</i>	–	1	1 (3%)
	<i>T. tonsurans</i>	–	–	1 (3%)
	<i>T. violaceum</i>	1	2	3 (10.5%)
	<i>T. schoenleinii</i>	1	1	2 (7%)
Geophilic (3%)	<i>M. gypseum</i>	1	–	1 (3%)
Total		12	17	29

Table 3. Number of *tinea unguium* positive cases by age in relation to females and males finger and toe nails

Age	Sex & involved nail			
	Female		Male	
	Finger nail	Toe nail	Finger nail	Toe nail
1 - 10	0	1	1	2
11 - 20	1	1	2	3
21 - 30	3	3	3	4
31 - 40	1	2	3	3
41 - 50	1	1	1	1
51 - 60	1	1	0	1
61 - 70	0	0	0	1
Total	7	9	10	15

quently than females (Table 1). Toe nails (59%) were infected more than fingernails (41%), but in final, *tinea unguium* in males were seen with more frequently than females [males 24 cases (4% total suspected subjects), females 17 cases (3% total suspected subjects)]. These results are summarised in table 3.

Proximal subungual *tinea unguium* secondary to leukonichia was seen in five cases (12% total positives). Primary leukonichia and the Lateral Subungual Distrophy and also total dystrophic *tinea unguium* had the most frequency [36 cases (88% total positive cases)]. Secondary proximal subungual *tinea unguium*, was mainly observed in finger nails (12 cases out of 17 cases), while other clinical forms of *tinea unguium* were seen with similar frequencies in both toe and finger nails. All the clinical forms of *tinea unguium* were more frequent in male.

With regard to the diffusion of *tinea unguium* between the sexes, a higher prevalence was observed in the toe nails of males [15 cases (63% positive toe nails, 37% total positive cases, 6.5% total males, 2.5% total patients)]. The distribution of diffusion in toe and finger nails, on the other hand, was almost equal in women (nine and seven cases respectively from 354 total suspected women);

however, a slightly higher rate was observed in percentage of toe nails (22% positive cases in toe nails and 17% in finger nails).

Discussion

The analysis of patients with suspected cases of *tinea unguium* is not simple because, even though mycelia filaments are sometimes found in the collected materials, the corresponding culture may be negative. This type of results will occur even if the specimens are collected and processed carefully with extensive pulverisation. There are a few reasons why this may be: for example, therapy has already been carried out, an area of the infected nail without viable hyphae was sampled or transporting of local antifungal material into media when the samples were cultured. Even for specimens with positive result by direct microscopic examination, a 30%-70% rate of negative culture has been reported by different studies [12]. On the other hand, the growth of different dermatophyte colonies may sometimes be observed, which can be difficult for the issue of speciation. In addition, culturing of both a non-dermatophyte with a dermatophytic may occur, therefore making it hard to determine the etiological role of each fungus in this situation [15,28]. It is also possible to observe the growth of a fungus that is not able to invade nail [12]. Therefore, the analysis and description of suspected *tinea unguium* and its etiological agents, are sometimes more difficult than in other forms of dermatophytosis, as demonstrated by the attempts of various authors to improve diagnostic methods or to redefine diagnostic criteria [1,11,18,19].

Seven percent of the subjects examined in this study for suspected *tinea unguium* proved to be infected. This rate is similar to that reported from Iran by other authors [21,27,32,33]. All other suspected cases had non-fungal problems such as psoriasis with psoriatic fingernails, nail trauma, eczema, and yellow nail syndrome for nail dystrophy.

In agreement between authors, also Iranian medical mycologists and dermatologists the most important etiological agents of *tinea unguium* are related to the zoophilic group according previous studies [7,21-23,27,30,33,34,40,43]. These studies have also reported that the highest frequency of *tinea unguium* is seen in the 11-40 years age group. The high prevalence of *tinea unguium* amongst younger subjects in comparison with the prevalence of this mycosis in other parts of the world could be related to the direct and indirect contact of young people with animals and their products (leather, wool) during animal husbandry and farm works, in addition to the existence of domestic pets (cat, dog) in the majority of homes in this region of Iran. The wearing for long periods of waterproof or full-length boots during animal husbandry and farm work could provide a suitable environment for the growth of the dermatophytes, because of the moisture and warmth around the toe.

Comparing our results with those from different countries, we found only two cases of *Trichophyton rubrum* infection, while other authors have found *T. rubrum* to be the principle or one of the main dermatophyte responsible for *tinea unguium*, followed by *Trichophyton mentagrophytes*. Also Aida et al. reported the frequencies of *T. rubrum* and *T. mentagrophytes* at 50% and 20% respectively, whereas Gupta et al. reported that *T. mentagrophytes* was the main etiological agent followed by *T. rubrum* in distal and lateral subungual and white superficial onychomycosis in toe nails [6,9,12,20,42,45].

Some rare cases by *Trichophyton tonsurans*, *Trichophyton equinum* and *Microsporum canis* have also been observed [28,33].

On the basis of some studies, it has been suggested that: a) *Tinea unguium* mainly infects toe nails and b) Certain species of dermatophytes have a specific tropism for toe or finger nails [26,45]. However, in this study, the frequency of each species of dermatophyte between toe and finger nails did not differ significantly. In another study, the infection started as a *tinea pedis* or *tinea corporis* and then involved toe nails [8]. Some authors and results supported the idea that, all dermatophyte species are probably capable of nail invasion [1,26]. Finally, it is probable that the incidence of dermatophyte species in *tinea unguium* reflects their prevalence in that region of the world. In fact, *T. mentagrophytes*, *Trichophyton verrucosum*, *M. canis* and *Trichophyton schoenleinii* are, by far, the most prevalent species in North-west Iran. In this study, *T. mentagrophytes* (31%) and *T. verrucosum* (21%) were the main etiological agents of *tinea unguium*, followed by *M. canis* (14%) and *Trichophyton violaceum* (10%) (Table 2). *Trichophyton rubrum* is not a native dermatophyte in Iran and in the other dermatophyte studies in Iran, this fungus has rarely been found [7,21-23,27,30,31,34,40,43].

Animal husbandry is one of the main economic activities in North-west Iran. Therefore, it is probable that direct and indirect contact with animals, such as sheep, cattle, horses and goats, and their products, such as leather and wool, are a risk factor for *tinea unguium*. Likewise, the existence of domestic pets such as cats and dogs, in the home could play a role in *tinea unguium*.

During the last decade, a world-wide (except USA) decrease [13,19] in anthropophilic dermatophytes has been reported, with a corresponding increase in zoophilic dermatophytes [25]. As a consequence, many cases of onychomycosis by various species of dermatophytes have been reported recently [2-4,8,13,14,23,35,40,43].

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