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Original article

Mycological Study of Superficial-cutaneous Mycoses in Tehran, Iran

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Abstract

Background: Many studies have been conducted on fungal infections which are known as public health and therapeutic problems. Since the prevalence rate of the fungal diseases and their etiological factors are changing over time, the purpose of this study was to identify the prevalence rate of superficial-cutaneous fungal infections (SCFIs) in order to understand the ways of their dissemination, to prevent diseases transmission, to eliminate contamination sources and predisposing factors, and to take appropriate action for their treatment.
Materials and Methods: After referral to medical mycology laboratory of Tehran University of Medical Science from 2014 to 2015, the patients were subjected to mycological examinations, and sampling of patients' lesions was performed. Directsmears were prepared with Potassium hydroxide. Samples were cultured on Sabouraud dextrose agar medium, and species were identified.
Results: From a total of 916 suspected patients, 334 cases (36.5%) had SCFIs. Dermatophytosis was the most prevalent SCFI (55.7%), followed by cutaneous candidiasis (19%), tinea versicolor (14.3%), and non-dermatophytic molds (11%).Tineapedis was the frequent site of involvement. *Trichophytonmentagrophytes* was the predominant species of dermatophytosis.
Conclusion: According to the obtained results on the prevalence rate of SCFIs between male and female patients in different age groups and also by taking into account the type of the prevalent fungi and the involvement site of the fungal infection, it is possible to take appropriate action for prevention and treatment of these kind of diseases by using important keys of the results to research etiological and underlying factors involved in these diseases.

Keyword: Superficial, Cutaneous, Fungal, Infection, Mycoses

1. Background

Despite numerous advances in health and medical sciences, superficial-cutaneous fungal infections (SCFIs) have retained their position as one of the most important skin diseases (1-3). Extremely high prevalence rate of SCFIs and their worldwide distribution have made them as one of the most common dermatological diseases. Causative agents mostly are consisted of *dermatophytes*, yeasts such as *Candida* and *Malassezia*, and non-*dermatophyte* molds (4). Many comprehensive studies have been conducted on SCFIs which are known as public health and therapeutic problems (5-9). According to these studies, the incidence of SCFIs depends on climatic and geographical conditions, and apart from factors such as age and host defense, it is influenced by individual and social behaviors (5-11). Predisposing factors such as diabetes, infections caused by viruses targeting human immune system, the use of immune suppressive therapy, cancer chemotherapy, antibiotics, and avid sports participation have essential role in increasing these infections every year (7, 12). The lack of personal hygiene which is considered as the primary mechanism of the diseases dissemination in every societycontributes to SCFIs and sometimes leads to itsepidemic in community centers such as schools, kindergartens, barracks, and prisons. Therefore, research on the prevalence rate of SCFIs for control and prevention of fungal diseases have an essential role in reducing skin diseases and public health problems (7, 12).

2. Objective

Surveying fungal infections has special importance in different communities because of their effect on public health. Since the prevalence rate of the fungal disease and their etiological factors are changing over time, the purpose of this study was to identify the prevalence rate of SCFI in order to use data from this study for further understanding the ways of dissemination and predisposing factors, and to take additional appropriate interventions for their treatment.

3. Materials and Methods

3.1. Sampling

This cross-sectional study was performed in medical mycology laboratory of school of health in Tehran University of Medical Sciences, Iran, from 2014 to 2015. After visiting by specialist physicians, the patients were referred to the laboratory for mycological examinations due to skin, nail, and hair lesions. The characteristics of the patients not using anti-fungal drugs and not taking baths in the last two days were registered in lab's book, and their sampling was done. Skin samples were collected by scraping the skin with a sterile scalpel. In the case of distal nail lesions, the sampling was done from deep parts and nail beds after removing the nail. In proximal nail lesions, the samples were collected from the depth of the nail, and in the white superficial onychomycosis cases, the samples were collected from the surface of the nails. In cases of

hair involvement, the infected hair shafts were removed. In suspected cases to tinea versicolor, scotch tape method was used for sampling.

3.2. Direct Microscopic Examination (DME)

Direct smears were prepared with 15% Potassium hydroxide (KOH) (in hair cases with lactophenol) from the collected samples, and in suspected cases to tinea versicolor, methylene blue staining was used for prepared smears, too. The prepared smears were examined by optical microscope (Olympus, Germany) for the presence of fungal elements (hypha, arthrospores, yeast cells, and pseudohyphae) after putting in the moisturous environment for an hour.

3.3. Culture and identification

Some of the samples were cultured by transplant method on SC (Sabouraud dextrose agar with 0.005% chloramphenicol) and SCC (Sabouraud dextrose agar with 0.05% cycloheximide and 0.005% chloramphenicol) media (E. Merck, Germany). The media were kept at25-28° C and checked twice weekly for the evidence of colony growth. No growth after 4 weeks of incubation was considered as negative for dermatophytes. This time for *Candida* and molds was 3 weeks. In order to identify *dermatophyte* and mould isolates, if there were any, colony morphology and microscopic examination with lactophenol cotton blue were used to observe hyphae structure and shape together with presence and arrangement of microconidia and macroconidia. Differential methods such as hair perforation test, *Trichophyton* nutritional media, urease test, temperature tolerance and temperature enhancement test, pigment production, Czapek's agar, or other selective media were used for the identification of some species whenever needed (12). Yeast isolates, if there were any, were identified by the use of standard laboratory methods including the germ tube test, morphology on corn-meal agar-tween 80 (CM-T80) using the dalmau method, chromagar *Candida* (Microbiology company, France), and API C20 Aux system (Bio Merieux, Marcy, 1 Etoile France). Each yeast should have represented a unique isolate from a patient; otherwise, it was placed and maintained as water suspension at room temperature in our laboratory for further use. *Malassezia* spp. infection was diagnosed based on direct examination.

3.4. Statistical analysis

The data analysis was performed by SPSS software version 18 (SPSS Inc., Chicago, IL). The study was assessed by using standard Chi-squared and fisher test with 95% confidence intervals (CI). *P*value < .05 was considered as statistically significant.

4. Results

From a total of 916 suspected patients, the existence of SCFIs was proved in 334 cases (36.5 %). In this study, dermatophytosis was the prevalent infection, and tinea pedis was the most frequent clinical form of dermatophytosis. There was a significant difference between the involved sites and the type of dermatophytosis (*P*< .05). *Trichophytonmentagrophytes* was the most common *dermatophyte* isolated from dermatophytic patients, and there was a significant difference between the type of isolated species and dermatophytosis (*P*< .05).The maximum prevalence rate of dermatophytosis was observed in the age group of40-49 years with causative agent of *T. mentagrophytes*, which was appeared as tinea pedis. But there was no significant difference between the age groups and the type of dermatophytosis (*P* > .05). In regard to the sources of the infection in dermatophytosis, anthropophilic fungi and zoophilic fungi accounted for 89.1 and10.9% of the dermatophytosis, respectively. No geophilic fungi were observed. About 35% of the patients with tinea pedis and onychomycosis were also diagnosed as having diabetes. In SCFIs, the most prevalence rate of the infections was observed in summer, and the least prevalence rate was observed in autumn. SCFIs were more prevalent in males than in females. However, infemales, cutaneous candidiasis and non-dermatophytic molds of onychomycosiswere more prevalent than in males. There was significant difference between the genus of patients and dermatophytosis, candidiasis, and non-dermatophytic molds of onychomycosis (*P*< .05). But in tinea versicolor, there was no significant difference between genus of patients and the disease (*P* > .05). In case of cutaneous candidiasis (63 cases, 19 %), *Candida albicans* was the most prevalent isolated species (60.3 %), followed by *C.parapsilosis* (22.3 %), *C.tropicalis* (12.7%), *C.guilliermondii* (3.1 %), and *C.krusei* (1.6 %). The most frequent lesion site of cutaneous candidiasis was observed in fingernails in all age groups. Non-dermatophytic molds of onychomycosis were detected in 37 patients (11%). Among them, *Aspergillus* spp. were found in 75.7 %, followed by *Fusarium* spp. (13.5 %), *Scopulariopsis* spp. (5.4 %), *Chrysosporium* spp. (2.7%), *Cladosporium* spp. (2.7 %). In this infection, prevalence rate order of *Aspergillus* species were *Aspergillus flavus, Aspergillus niger,* and *Aspergillus terreus,* respectively. In non-dermatophytic molds of onychomycosis, toenail involvement was more than fingernail. Table1 shows absolute and relative frequency of SCFIs based on patients' genus. Table2 shows the frequency of culture isolated *dematophytes* species based on different types of tinea (only culture positive dermatophytosis). Table3 shows the frequency of different types of tinea (positive direct microscopic examination and culture positive dermatophytosis) based on age group and genus. Figure1 illustrates the frequency of onychomycosis in patient with non-dermatophytic molds based on age group and genus.

5. Discussion

One of the most common mycoses diseases is SCFI by which a great number of people get infected annually. The prevalence rate of SCFIs in 20-25% of the world's population illustrates the importance of these infectious diseases which are known as zoonotic diseases, too (11). In the current study by taking into account the obtained results, it was determined that from 916 suspicious patients , 334 cases (36.5%) were actually infected by SCFI, and dermatophytosis was the most common infection (55.7%),followed by cutaneous candidiasis (19%),tinea versicolor (14.3%), and non-dermatophytic molds of onychomycosis (11%), respectively (Table1). Studies conducted in Iran have reported the dermatophytosis prevalence rate - among other SCFIs– to be between 10.8 to 76.9%(5, 7,12),and worldwide studies have reported the dermatophytosis prevalence rate- among other SCFIs- from 13.8% in Spain to 88.3% in Japan(13, 14).

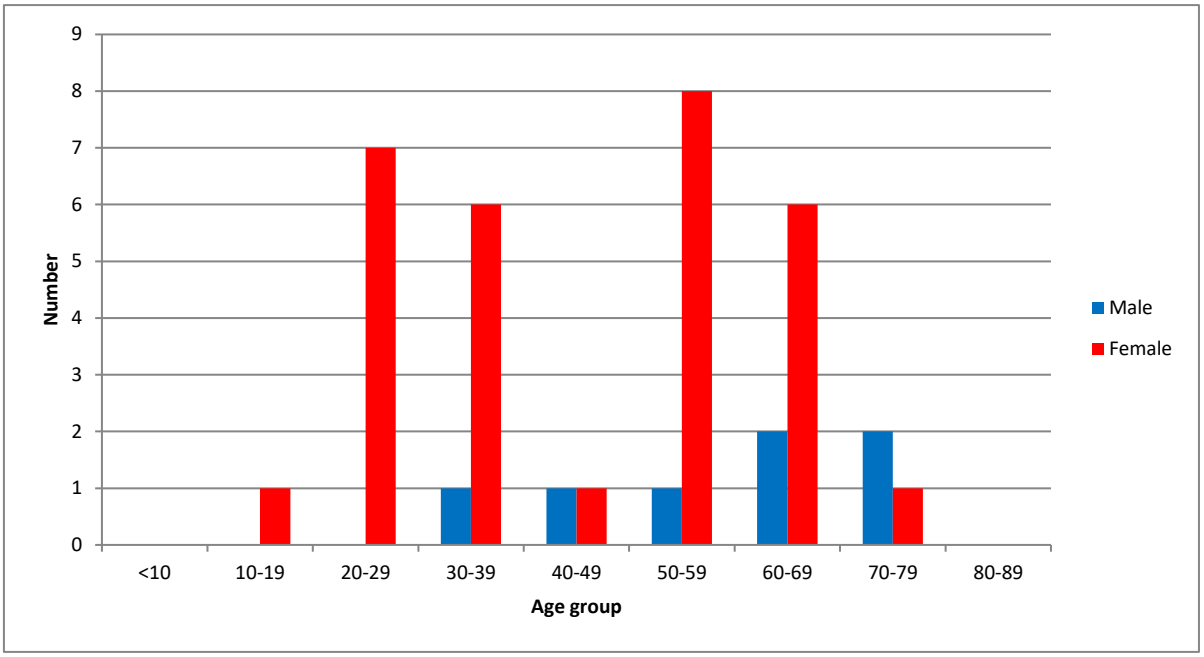

Figure 1. Frequency of onychomycosis in patient with non-dermatophytic molds based on age group and genus.

Table 1. Frequency of superficial-cutaneous fungal infections based on genus.

	Male No. (%)	Female No. (%)	Total No. (%)
Dematophytosis	152 (72)	34 (27)	186 (55.7)
Candidiasis	19 (9)	44 (36)	63 (19)
Tinea versicolor	32 (15)	16 (13)	48 (14.3)
Non-Dematophytic molds	7 (4)	30 (24)	37 (11)
Total	210 (63)	124 (37)	334 (100)

No.: Number, (%): Percentage

Table 2. Frequency of culture isolated dematophyte species based on different type of tinea (Dermatophytosis).

Type of tinea/ Dermatophyte species	T. pedis No. (%)	T. unguium No. (%)	T. corporis No. (%)	T. cruris No. (%)	T. manuum No. (%)	T.faciei No. (%)	T.capitis No. (%)	Total No. (%)
T. mentagrophytes	25 (21)	13 (11)	3 (2.5)	4 (3.35)	3 (2.5)	0	0	48 (40.3)
T.rubrum	15 (12.6)	8 (6.7)	6 (5)	3 (2.5)	0	1 (0.85)	0	33 (27.7)
T. tonsurans	0	0	5 (4.2)	0	1 (0.85)	4 (3.35)	3 (2.5)	13 (10.9)
T. verrucosum	1 (0.85)	1 (0.85)	2 (1.7)	0	5 (4.2)	2 (1.7)	0	11 (9.2)
E. floccosum	0	0	1 (0.85)	9 (7.55)	0	0	0	10 (8.5)
T. violaceum	0	1 (0.85)	1 (0.85)	0	0	0	0	2 (1.7)
M. canis	0	0	2 (1.7)	0	0	0	0	2 (1.7)
Total	41 (34.5)	23 (19.3)	20 (16.8)	16(13.45)	9 (7.55)	7 (5.9)	3 (2.5)	119 (100)

T: Tinea, No.: Number, (%): Percentage

Table 3. Frequency of dermatophytosis based on age group and genus

Type of tinea / Age group	T. pedis No. (%)	T. unguium No. (%)	T. cruris No. (%)	T. corporis No. (%)	T. manuum No. (%)	T. faciei No. (%)	T. capitis No. (%)	Total No. (%)
< 10	0	0	0	8 (27.5)	0	2 (22.2)	2 (66.6)	12 (6)
10-19	1 (1.5)	0	2 (6.6)	7 (24.1)	4 (28.5)	4 (44.4)	1 (33.4)	19 (10.2)
20-29	5 (7.6)	0	11 (36.7)	5 (17.3)	3 (21.5)	1 (11.2)	0	25 (12.3)
30-39	10 (15.2)	2 (5.8)	7 (23.5)	5 (17.3)	2 (14.3)	0	0	26 (14)
40-49	19 (28.8)	12 (34.2)	2 (6.6)	2 (6.9)	0	2 (22.2)	0	37 (19.9)
50-59	12 (18.1)	7 (20)	2 (6.6)	2 (6.9)	3 (21.5)	0	0	26 (14)
60-69	11 (16.6)	6 (17.2)	5 (16.7)	0	2 (14.2)	0	0	24 (15)
70-79	6 (9.2)	5 (14.3)	1 (3.3)	0	0	0	0	12 (6)
80-89	2 (3)	3 (8.5)	0	0	0	0	0	5 (2.7)
Total	66 (100)	35 (100)	30 (100)	29 (100)	14 (100)	9 (100)	3(100)	186 (100)

T: Tinea, No.: Number, (%): Percentage

In other studies conducted in Japan in 2002 and in Turkey from 2000 to 2007, dermatophytosis was the most prevalent SCFI, followed by cutaneous candidiasis and tinea versicolor, respectively (14, 15). These results on the SCFIs prevalence rate, are in accordance with the current study's results which are also in accordance with the other studies conducted in Iran such as Sadeghi et al. (2011) in Tehran (5), Nasrollahi Omran et al. (2009) in Tehran (8), Khazaiee et al. (2010) in Arak (16), and Aziziet al. (2001) in Yazd (17). In all of them, dermatophytosis has been reported to be the most prevalent infection. In the present study, male individuals were more infected by SCFI than women(Table1); this finding is in accordance with the other studies conducted by Sadeghiet al. (5), Zamani et al. (7), Nasrollahi Omran et al. (8), Bassiri-Jahromi et al. (18, 19) in Tehran and other regions of Iran (16, 17, 20, 21) and other countries(13, 22). However, the difference in genus affection can be influenced by other factors such as personal hygiene, occupational factors, and exposure to the contamination. In the present study, tinea pedis, tinea unguium, tinea cruris, and tinea corpor is had the most prevalence rate, respectively (Table3). The order of the tinea prevalence rate in this study is in line with some studies (9, 19); but different from others (5, 7, 13, 17, 21, 22). However, in some of these studies such as Sadegi et al. (5) and Zamani et al. (7) studies, similar to the current study, tinea pedis was the predominant type of dermatiphytosis. Difference in prevalence rate of tinea types could be attributed to such factors as dissemination of the pathogenic species, social habits and customs, individuals' activity pattern, weather conditions, and personal and social hygiene of the persons inhabited in different regions. The predominant causative species of dermatophytosis was *T.mentagrophytes* (Table2); this result is also in line with the other studies conducted in Tehran (7, 9, -22), Isfahan (23), and Ahwaz (24). However, in this case, it is not in accordance with the studies in which *T.rubrum*has been reported as the most common species (5, 25-28). The difference between the obtained results of the current study and the other studies could be attributed to such factors as, time and location conditions, migrations and travels, cultural and social pattern, life styles, and etc. According to this study, it was determined that most of the infections related to the dermatophytosis have been caused by anthropophilic species of *dermatophytes*, which are commonly observed in social life. Urban societies were involved with these species more than other regions, and the dissemination of the infection was more in areas with low health standards than the other.

Candidiasis is one of the most common opportunistic mycosis diseases, and in the present study with the prevalence rate of 19% was in the second rank of SCFIs (Table1). The most common clinical form of cutaneous candidiasis was onychomycosis with 84%. The prevalence rate of *Candida* onychomycosis in women was more than in men; these finding is consistent with the finding of the other studies conducted by Razaghi-Abyaneh et al. (6), Sadeghi et al. (5) in Tehran and other researchers (12). This type of onychomycosis is more prevalent in individuals keeping their hands under water for a long time such as housewives, nurses, servants, and dishwashers. This disease occurs in children due to sucking fingers. In this study, the most common onychomycosis agent was *C.albicans* with 60.3%; this finding is also similar to other studies conducted in Tehran (5-6, 29, 30) and other regions of Iran and the world (31-35). However, it's not in accordance with some studies conducted in Tehran (36) and other regions (37, 38), in which *dermatophytes* have been reported to be the main causative agents of onychomycosis. The most cases of *Candida* onychomycosis were observed in fingernails, which could be due to hormonal differences or women's more working at house and constant contact with water and detergents. In Razaghi-Abyaneh et al. (6), Sadeghi et al. (5), Hashemi et al. (30) and Zeini et al. (39) studies, the infection was also more prevalent in fingernails than in toenails.

In this study, the onychomycosis caused by non-dermatophytic molds was more prevalent in toenails. This finding is similar to other studies conducted by Sadeghi et al. (5), Geramishoaret al. (29), Ahmadi et al. (40), Hashemi et al. (30), Zeini et al.(39), Hilmioglu et al.(41), Veles et al. (42), and

Ginni et al.(43). Toenail involvement with saprophytes could be attributed to the fact that toenails are more susceptible to trauma, and trauma as a predisposing factor provides the basis for penetration of these soil resident saprophytic fungi in the nails. In this study just like to the previous studies conducted by Sadeghi et al. (5), Hashemi et al. (30), Zeini et al. (39) in Tehran and by Agarwalla et al. in Nepal (44), the most common non-dermatophytic mold isolated from nails was *Aspergillus* genus. Similar to Sadeghi et al. study (5), the high prevalence rate of non-dermatophytic molds of onychomycosis in this study was in the age group of 50-59 years.

Tinea versicolor is widespread and more prevalent in tropical and sub-tropical climates. Given reports from different climatic regions of Iran and other parts of the world are different. In the current study, tinea versicolor present in 14.3% of all the patients diagnosed with SCFIs was in the third order. It was more prevalent in men than in women and often observed in chest (37.6%) and neck (33.2%) in the age group of 20-29 years. In NasrollahiOmran's study (8), this disease was equally prevalent in both gender and more prevalent in the age group of 20-29 years.

6. Conclusion

A wide spectrum of infectious dermatoses was highly prevalent among the population under study. The prevalence rate of superficial-cutaneous fungal infections has changed during recent years. There are lots of predisposing factors affecting epidemiology of these infections, including geographic area, hygiene, occupation, climate, contact with animals, genus, etc. According to the obtained results of the current study on the dissemination and incidence of SCFIs between male and female patients in different age groups and also by considering the type of the prevalent fungi and the prevalent involvement site of the fungal infection, it may be possible to perform appropriate intervention for prevention and treatment of these diseases and to investigate etiological and underlying factors involved in these diseases.

Conflict of interests

There was no conflict of interest in the present study.

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Authors' Contribution

All of the authors contributed to this study.

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