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Epidemiology of dermatomycoses in Kerman province, southeast of Iran: A 10-years retrospective study (2004–2014)

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ABSTRACT

Objectives: Superficial and cutaneous fungal infections (SCFIs) are an important public health problem and are common in tropical and subtropical countries. Pityriasis versicolor, dermatophytosis, erythrasma, onychomycosis, and otomycosis are the major diseases associated with SCFIs. The aim of this study was to evaluate the prevalence and causative agents of dermatomycoses over a period of 10 years in Kerman province, southeast of Iran.

Methods: A number of 1782 subjects, including 1096 females and 686 males, with cutaneous disorders in their skin, nail, and hair suspected to have SCFIs participated in this study. The collected specimens were examined using direct microscopy examination, staining, culture on specific media and PCR-RFLP technique.

Results: In total, 617 (34.62%) subjects had SCFIs, of whom 290 (47%) were female and 327 (53%) were male. Identified SCFIs included yeast infections, dermatophytosis, saprophyte onychomycosis, erythrasma, and otomycosis due to non-dermatophytic molds (NDMs). The highest prevalence of dermatomycoses was found among the 41–50-year and 31–40-year age groups. Tinea unguium was the most common clinical pattern of dermatomycoses, and *T. mentagrophytes* was the predominant agent. Also, *Aspergillus* spp. were the most common NDMs agents of onychomycosis and otomycosis.

Conclusions: This study summarized the epidemiological trends and etiologic agents of SCFIs in a 10-year period in Kerman, southeast of Iran. Consideration of the current epidemiologic trends in the prevalence and knowledge of the exact causative agents of SCFIs may play an important key role towards further investigations, diagnosis, and modification of current treatments.

1. Introduction

Cutaneous mycoses are common fungal diseases and are known as one of the important causes of morbidity in tropical and subtropical countries [1,2]. Pityriasis versicolor (PV), dermatophytosis, erythrasma, onychomycosis, and otomycosis are the major diseases associated with SCFIs. PV is a prevalent and mild disease that is characterized by a skin eruption on the trunk and proximal extremities, and is caused by lipophilic yeasts of the genus *Malassezia* [3,4]. *Malassezia* spp. are the etiological agents of hyper or hypopigmentation disorders, catheter-associated fungemia, folliculitis, seborrheic dermatitis, dacrylitis, blepharitis, onychomycosis, as well as nosocomial bloodstream infections in pediatric care units [5].

Yeast infections result from an overgrowth of yeast species in any part of the body. Candidiasis is a fungal infection due to different *Candida* species and is the major common type of yeast infections [6–9]. The non- *Candida* yeasts include *Cryptococcus*, *Trichosporon, Rhodotorula, Malassezia*, and *Sporobolomyces* species. These yeasts are considered as causative agents of mucosa-associated, superficial infections and disseminated infections in immunocompromised patients [10–12].

In cutaneous mycoses, *dermatophyte* spp. attach to the integument and its associated structures, including nails, hair and rarely, the deeper layers of the epidermis [13]. Dermatophytes are comprised of three genera of *Epidermophyton*, *Microsporum*, and *Trichophyton* [14,15]. Dermatophytosis has a worldwide

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List of abbreviations

SDA	Sabouraud Dextrose Agar
SCFIs	Superficial and cutaneous fungal infections
PV	Pityriasis versicolor
KOH	Potassium Hydroxide
PCR-RFLP	Polymerase Chain Reaction Restriction Fragment Length Polymorphism
NDMs	Nondermatophytic molds
CI 95%	Confidence Interval 95%
OR	Odds Ratio

distribution [16]. Different types of dermatophytosis include tinea cruris (jock itch), tinea pedis (athlete's foot), tinea manuum, tinea unguium or onychomycosis, tinea capitis (ectothrix/endothrix/favus), tinea corporis, tinea barbae, tinea faciei, and allergy to dermatophytes [14,15,17]. Transmission of dermatophytes occurs by direct contact with infected people (anthropophilic organisms), infected animals (zoophilic organisms), and infected soil (geophilic organisms) [18,19]. The aim of this study was to evaluate the prevalence and causative agents of dermatomycoses over a period of 10 years in Kerman province, southeast of Iran.

2. Materials and methods

2.1. Subjects and samples collection

This study included 1782 subjects with cutaneous disorders in their skin, nail, and hair suspected to be SCFIs, who were admitted to the medical mycology laboratory of the Afzalipoor Faculty of Medicine in Kerman, Iran, from March 2004 to March 2014. These subjects had been forwarded by dermatologists to the medical mycology laboratory. This laboratory serves as a reference lab for the entire population of the province. To complete the epidemiological data, general patient information such as age, sex, personal health status, residence (classified as rural and urban), occupation, socioeconomic status, underlying physiology or immune status, previous treatments, family and personal history, chronic dermal diseases, disease duration, lesion area, clinical findings, and clinical type of lesion was recorded. The subjects that received topical and/or systemic antifungal agents for at least seven days or corticosteroid agents for three weeks were excluded prior to the sample collection. This study was approved by the ethics committee of the Kerman University of Medical Sciences (K/92/589). Written informed consent was provided by the participants or in the case of children, by their parents to use their clinical records for the study. Specimens including skin scrapings, nail clippings, and hair were obtained from various sites on the suspected patient's body based on clinical symptoms in the following locations: body, hand, foot, groin, trunk, face, head, scalp, toenail, and fingernail.

2.2. Identification of fungal strains

All the specimens were tested for fungal identification using mycological testing methods, including direct microscopy examination, staining, culture on specific media and PCR-Restriction Fragment Length Polymorphism technique.

2.2.1. Direct microscopy examination and staining

The different clinical specimens (hair, skin and nail scales) were examined upon treatment with 10–20% potassium hydroxide (KOH, Merck, Germany). Also, the skin lesions on the patients' body were examined using a Wood's lamp for viewing colored fluorescence [20,21]. All the clinical specimens were evaluated using light microscopy in 400× of magnification, and the presence of the fungal structures such as arthroconodia, blastoconidia, true hyphae, pseudohyphae, and conidia was investigated [22].

2.2.2. Culturing on specific media

A portion of the different collected clinical specimens (hair, skin and nail scales) was cultured on Sabouraud dextrose agar (SDA, Merck, Germany), SDA supplemented with chloramphenicol (50 mg/mL) covered with a thin layer of sterile olive oil, modified Dixon agar (Merck Co., Darmstadt, Germany), and Mycosel agar (Merck Co., Darmstadt, Germany) [23–25]. The plates were incubated aerobically at 30 °C for 30 days and checked weekly. The plates without growth after 30 days were considered to be negative. The *dermatophyte* species were identified using their macro- and microscopic characteristics, growth on dermatophyte test medium, urease test, and hair perforation test. NDMs were identified based on colony characteristics and microscopic morphology on Czapek Dox Agar (HiMedia, Mumbai, India). Identification of yeast isolates was performed based on morphology in Corn meal agar medium (QLAB, United Kingdom), CHROMagar *Candida* medium (HiMedia, Mumbai, India), and the germ tube test.

2.2.3. DNA extraction and molecular identification of isolated fungi using PCR-RFLP

In this study, PCR-RFLP technique with specific restriction enzymes was used for identification of isolated fungi. Briefly, DNA was extracted from each of the fungal culture using Exgene Tissue SV Plus-mini kit (Gene All, General Bio System, Seoul, South Korea) based on the manufacturer's instruction. PCR amplification was performed using the universal fungal primers (ITS1: 5′-TCC-GTA-GGT-GAA-CCT-GCG-G-3′ and ITS4: 5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3′) [26]. RFLP was done according to the manufacturer's instructions using *Msp I* (Fermentas Life Sciences, Lithuania), *Mva I* Fast digest (Fermentas Life Sciences, Lithuania) and *Mwo I* (Fermentas Life Sciences, Lithuania) for identification of different *Candida* spp. [23], *dermatophyte* spp. [27] and *Aspergillus* spp. [28], respectively.

2.2.4. Mycological diagnose criteria

Since some filamentous fungi such as *Aspergillus* and *Fusarium* species and also, yeast-like fungi such as *Candida* spp. have colonized the skin surface is an inoffensive form, therefore, the isolation of these fungi should be interpreted with caution. These fungi are able to colonize or infect patients, depending on the integrity of the immune system. For report of the disease, the observation of fungal structures in direct microscopy examination coupled with positive cultures of samples is required. The other criteria of approving the existence of the disease were the positive mycological examination (direct microscopy examinations and culture) during two different occasions (different days of sampling) and observation of colonies growth at seven equidistant positions seeded to the surface of the petri dishes. Also, immune status and occupation of individuals should be considered [22,29].

2.3. Statistical analysis

Analysis of data was performed using SPSS (Statistical Package for Social Sciences) version 21. To account for any relationship between the variables, chi-square test, Fisher's exact test, and whenever required, Odds Ratio and respective confidence interval were used. A p-value of <0.05 was considered significant.