JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2018. Vol. **12**(3), p. 1215-1219

Etiologic Diversity of Onychomycosis in Mexican Patients with Chronic-Degenerative Diseases

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http://dx.doi.org/10.22207/JPAM.12.3.22

(Received: 01 June 2018; accepted: 19 July 2018)

Onychomycosis is the most common nail disease caused by dermatophytic fungus, non-dermatophytic molds or yeasts. It is an infection that continues to increase due to various factors such as chronic degenerative diseases. The aim of this study was to evaluate the etiologic diversity of onychomycosis in Mexican patients with chronic-degenerative diseases. The research was performed in 51 adult outpatient of a second level hospital in the city of Puebla, Mexico. Isolation and fungal identification were executed by using conventional methods (Microculture, Auxacolor, CHROMagar-Candida). All patients had onychomycosis on the toenails. Type 2 diabetes mellitus was the most common chronic degenerative disease. According to the richness and abundance of organisms, we found: *Candida albicans* 17% (9), *Candida glabrata* 6% (3), *Trichophyton mentagrophytes* 8% (4), *Trichophyton rubrum* 4% (2), *Trychophyton tonsurans* 6% (3), *Microsporum canis* 6% (3), *Scopulariopsis brevicaulis* 12% (6), *Fusarium solani* 19% (10), *Penicillium* sp. 8% (4) and *Aspergillus flavus* 14% (7). A high percentage of non-dermatophyte filamentous fungi isolates was obtained indicating the importance of these fungi as causal agents of onychomycosis in this population.

Keywords: Onychomycosis, Etiologic diversity, Chronic-degenerative diseases, Non-dermatophytic molds, dermatophytes.

The term onychomycosis comes from the Greek where onychos means nail and fungal infection mycosis. It is a growing problem of global health and the most frequent disease of the nails; it corresponds to more than 50% of the onychopathies[1-3]. Its prevalence is increasing worldwide, from 2.1% to 9.1%. In Mexico, onychomycosis accounts for 24% of this group of mycoses and 50% of all nail conditions. From this percentage, 90% corresponds to the toenails, being the main etiological agents the dermatophytes such as: *Trichophyton rubrum*, yeasts of the genus *Candida* spp, presenting a higher prevalence in the nails of hands, and also we find non-dermatophytic molds, such as *Fusarium* spp, *Aspergillus* spp, *Scopulariopsis* spp and *Acremonium* spp, as responsible for 2% to 20% of the isolates in clinical samples of nails, being its interpretation as causal agent of the frequently complicated nail alteration. The latter two groups usually invade secondary nail diseases or trauma, while dermatophytes may cause primary infections[4-9].

Onychomycosis continues to increase despite improvements in quality of life and personal hygiene measures. Although they may also be caused by the use of occlusive footwear, smoking, regular swimming, manicure and pedicure[10,11]. It is very often transmitted by walking

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barefoot on moist soil in areas such as; gyms, locker rooms, saunas and swimming pools[12,13]. Underlying conditions such as tinea pedis (athlete's foot), nail damage and nail psoriasis may contribute to the risk of onychomycosis, as well as advanced age. About 18.2% of the elderly patients present with this infection, compared to 0.7% in patients younger than 19 years; being men up to three times more susceptible to onychomycosis than women, the reason for this gender difference is unclear. Currently, in many countries, including Mexico, chronic degenerative diseases, such as cancer, diabetes, AIDS and circulatory problems, are the predominant conditions among the elderly and contribute greatly to the presence of onychomycosis, since the immune system offers less resistance. Therefore, susceptibility to infection increases[14,15]. Moreover, onychomycosis may be an important antecedent for the development of foot syndrome and diabetic foot ulcers[16-18]. Due to the importance of this problematic in this study it was proposed to determine the etiological diversity of onychomycosis in patients with chronic degenerative diseases.

MATERIALS AND METHODS

Study design and patients

A cross-sectional, descriptive and observational study was carried out, including 51 adult patients, who were treated at the outpatient clinic of a second level hospital in the city of Puebla. The population of this study included subjects with onychomycosis with different ages and both sexes, the epidemiological characteristics of the patients were recorded and informed consent was obtained.

Specimen collection

A total of 51 samples were obtained, one sample from each patient included in the study. To obtain each sample, the affected area was previously cleaned with ethanol (70%) to remove bacterial microbiota, exudation or excipients from previous treatments that may make it difficult to observe the sample and culture it. Sampling was performed through scraping and obtaining nail fragments, the products obtained were placed in a sterile Petri dish, labeled with corresponding data.

Direct examination of the samples

A portion of the sample was used for direct examination with 15% potassium hydroxide solution (KOH) (Emsure). It was allowed to act for 5 to 10 minutes for sample clearance. Then, a glass slide containing the sample and 20% KOH was examined using light microscopy (Zeiss) to identify the existence of fungal elements, including arthrospores, segmented hyphae, and or pseudohyphae and yeast cells.

Mycological identification

Another part of the sample was seeded at three equidistant points on Sabouraud Dextrose Agar (Bioxon) added with antibiotics (chloramphenicol and cycloheximide) and without antibiotics. The cultures were incubated at a temperature of 26 °C ± 2 °C for a maximum period of 15 days, checked daily until growth was observed[19]. The developed fungi were stained with cotton blue and lactophenol for microscopic observation to continue reseeding and obtaining axenic cultures. Also, micro-cultures of the filamentous fungi obtained were carried out and restorations were performed on Dermatophyte Test Medium (DTM) (BD). Identification of the dermatophytes species was performed based on colony morphology, color of colonies (surface and reverse), macro- and microscopic characteristics by slide culture procedure.

Growth of yeasts and physiological characterization

Another part of the sample was seeded at three equidistant points on Sabouraud Dextrose Agar (Bioxon) added with antibiotics (chloramphenicol and cycloheximide) and without antibiotics. The cultures were incubated at a temperature of 35 °C ± 2 °C for a maximum period of 7 days, checked daily until growth was observed[20]. The developed fungi were stained with cotton blue and lactophenol for microscopic observation to continue reseeding and obtaining axenic cultures. Isolated yeasts were identified phenotypically, physiological tests, chlamydoconid production, serum filamentation and thermotolerance, AUXACOLOR and CHROMagar-Candida[21].

Data analysis

The species richness (the number of species present) and species abundance (the number of individuals per species) were calculated. The statistical analysis was performed by descriptive analysis of the data using the Statgraphics plus program 5.1. The variables were calculated with their absolute frequency and percentage. Kruskal-

Wallis test was performed on the biodiversity indexes and the level of statistical significance used was $P < 0.05$.

RESULTS

Clinical and epidemiological data of patients

A total of 51 patient samples were obtained of subjects with onychomycosis, with an onychomycosis duration of 1 to 15 years (average 4.6 years), 45% (23) of the patients were women and 55% (28) were men, with a range of age 18 to 85 years and an average of 55.11 years. 53% (27) of the total of individuals is from rural origin and 47% (24) from urban origin. With farmer occupations 48% (24), household 27% (14), factory workers 21% (11) and student 4% (2). All patients presented onychomycosis on the thumb of the feet, 57% (29) on the left foot and 43% on the right foot. The most frequent concomitant diseases associated with onychomycosis were diabetes mellitus 74%, diabetes mellitus with hypertension 8%, diabetes mellitus with liver failure 4%, liver failure 2%, arterial hypertension 8%, diabetes mellitus associated with hypertension and chronic renal failure 4% (28). The frequency of clinical forms were distal subungual onychomycosis 67% (34), proximal subungual onychomycosis 10% (5), superficial white onychomycosis 17% (9) and total dystrophic onychomycosis 6% (3).

Fungal diversity

The wealth and abundance of organisms were determined and found dermatophytes 27%, non-dermatophytes 49% and yeasts 24%, corresponding to: *Candida albicans* 17% (9*), Candida glabrata* 6% (3), *Trichophyton mentagrophytes* 8% (4*), Trichophyton rubrum* 4% (2), *Trichophyton tonsurans* 6% (3), *Microsporum canis* 6% (3), *Scopulariopsis brevicaulis* 12% (6), *Fusarium solani* 19% (10*), Penicillium* sp. 8% (4) and *Aspergillus flavus* 14% (7).

DISCUSSION

For many years the etiology of onychomycosis was focused only on dermatophytes and yeasts, however, as of 1980, reports of infections by various filamentous fungi increased markedly[4,22]. From the samples analyzed in this study, 27% correspond to dermatophytes, 24% to yeasts and a high percentage (49%), to non-dermatophyte molds; which shows that onychomycosis by non-dermatophyte filamentous fungi has been increasing in our population. In the research of Salas-Campos *et al*., (2009) clinical cases of onychodystrophy were reviewed, where all patients presented onychomycosis due to non-dermatophytic fungi of different genera; which coincides with our results[23]. In another study conducted in a third level hospital in Mexico City by Méndez Tovar *et al*., (2016) identified non-dermatophyte fungi, which coincides with our results, where we also identify variability of non-dermatophytic molds[24]. Interestingly, the most prevalent fungus was *Penicillium* sp., while in our study we also identified *Penicillium* spp. in 8% of cases, which demonstrates that non-dermatophyte fungi can be etiological agents of onychomycosis in individuals with some comorbidity. Also, our study in patients with chronic degenerative diseases coincides with the work of Sandoval *et al*., (2014) on the incidence of onychomycosis in patients with chronic renal failure and hemodialysis; where these patients developed infection linked to comorbidity due to lack of hygiene and medical care[25,26]. The most frequent disease in this study related to onychomycosis was diabetes mellitus. *Candida albicans* and *Candida glabrata* were also isolated as etiological agents causing onychomycosis in the patients studied, although these yeasts are generally reported as commensals[27,28]. In patients with chronic degenerative diseases, where patients are in immunosuppressive conditions may behave as pathogens[29].

CONCLUSIONS

The high frequency of onychomycosis found in patients with chronic degenerative diseases indicates that this is an important factor for the development of this condition of the nails.

Dermatophytes were isolated and identified; *Trichophyton rubrum, Trichophyton mentagrophytes* and *Trichophyton tonsurans* and *Microsporum canis,* yeasts; of the genus *Candida* as: *Candida albicans and Candida glabrata* and *non-dermatophyte* molds *Scopulariopsis brevicaulis*, *Fusarium solani*, *Penicillium sp*. and *Aspergillus flavus*.

The most isolated fungal agent was *Fusarium solani* non-dermatophyte mold fungus. It is important to note that non-dermatophyte fungi were isolated in a high percentage compared to those already reported. Onychomycosis was present in a higher percentage in men than in women, in individuals with agricultural occupation, and in the majority of the patients was found in the thumb of the left foot. The frequency of chronic degenerative diseases associated with onychomycosis were; diabetes mellitus, more frequently; followed by hypertension, liver failure and chronic renal failure.

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