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

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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. 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.

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. It is very often transmitted by walking...
barefoot on moist soil in areas such as; gyms, locker rooms, saunas and swimming pools\textsuperscript{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\textsuperscript{14,15}. Moreover, onychomycosis may be an important antecedent for the development of foot syndrome and diabetic foot ulcers\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 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-dermatophyte 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 markedly4,22. 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 results23. 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 molds24. 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 care25,26. 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 commensals27,28. In patients with chronic degenerative diseases, where patients are in immunosuppressive conditions may behave as pathogens29.

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 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.

**REFERENCES**

1. López-Jodra, O., Torres-Rodriguez, J.M. Especies fúngicas poco comunes responsables de onicomicosis. *Rev. Iberoam. Micol.* 1999; 16, 11-15.
2. Ghannoum, M., Isham, N. Fungal nail infection (onychomycosis): a never-ending story?. *PLoS Pathog.* 2014; 10, 1-5.
3. Méndez, T.L.J., Manzano, G.P., Rangel, B.R, Silva, G.I., Hernández, H.F., López, M.R. Frecuencia de onicomicosis por hongos filamentosos no dermatofitos en un hospital de tercer nivel. *Dermatol. Rev. Mex.* 2013; 57, 235-239.
4. Arenas, R. Dermatofitosis en México. *Rev. Iberoam. Micol.* 2002; 19, 63-67.
5. Nazar, J.R., Gerosa, P.E, Diaz, O.A. Onychomycoses: epidemiology, causative agents and assessment of diagnostic laboratory methods. *Rev. Argent. Microbiol.* 2012; 44, 21-25.
6. Alvarado, A., Hernández-Alvare, G., Fernández, R., Arenas, R. Onicomicosis por Candida en las uñas de las manos. *Dermatol. Rev. Mex.* 2014; 58, 323-330.
7. Manzano-Gayosso, P., Méndez-Tovar, L.J., Arenas, R., Hernández-Hernández, F., Millán-Chiu, B., Torres-Rodriguez, J.M., Cortés-González, E., Fernández, R., López-Martínez, R. Onychomycosis-causing yeasts in four Mexican dermatology centers and their antifungal susceptibility to azolic compounds. *Rev. Iberoam. Micol.* 2011; 28, 32-35.
8. Ranawaka, R.R., Nagahawatte, A., Gunasekara, T.A. Fusarium onychomycosis: prevalence, clinical presentations, response to itraconazole and terbinafine pulse therapy, and 1-year follow-up in nine cases. *Int. J. Dermatol.* 2015; 54, 1275-1282.
9. Yasemin, O., Qoraan, I., Oz, A., Balta, I. Prevalence and epidemiology of tinea pedis and toenail onychomycosis and anti fungal susceptibility of the causative agents in patients with type 2 diabetes in Turkey *Int. J. Dermatol.* 2017; 56, 68-74.
10. Mendoza, N., Palacios, C., Cardona, N., Gómez, L.M. Onicomicosis: afeción común de difícil tratamiento. *Rev. Asoc. Colomb. Dermatol.* 2012; 20, 149-158.
11. Fuentes-Rivera, D. Epidemiología y diagnóstico clinicoetológico de onicomicosis en un centro médico Universitario (junio 97- mayo 99). *Dermatol. Perú* 2000; 10, 21-33.
12. Tosti, A., Elewski, B.E. Onychomycosis: practical approaches to minimize relapse and recurrence. *Skin Appendage Disord.* 2016; 2, 83-87.
13. Sigurgeirsson, B., Steingrimsson, O. Risk factors associated with onychomycosis. *J. Eur. Acad. Dermatol. Venereol.* 2004; 18, 48-51.
14. Fukunaga, A., Washio, K., Ogura, K., Taguchi, K., Chiyomaru, K., Ohno, Y., Masaki, T., Nagai, H., Nagano, T., Oka, M., Nishigori, C. Onychomycosis as a warning sign for peripheral arterial disease. *Acta. Derm. Venereol.* 2013; 93, 747-748.
15. Wijesuriya, T.M., Kottahachchi, J., Gunasekara, T.D.C.P., Bulugahapitiya, U., Ranasinghe, K.N.P., Neluka, F.S.S., Weerasekara, M.M. *Aspergillus* species: An emerging pathogen in onychomycosis among diabetics. *Indian J. Endocrinol. Metab.* 2015; 19, 811-816.
16. Boyko, E.J., Ahroni, J.H., Cohen, V., Nelson, K.M., Heagerty, P.J. Prediction of diabetic foot ulcer occurrence using commonly available clinical information: the Seattle Diabetic Foot Study. *Diabetes Care* 2006; 29, 1202-1207.
17. Lavery, L.A., Armstrong, D.G., Wunderlich, R.P., Tredwell, J., Boulton, A.J. Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. *Diabetes Care* 2003; 26, 1435-1438.
18. Minaric, M., Kalenic, S., Vukelic, M., Syo, D., Belicza, M. Candida infection on diabetic foot ulcers. *Diabetol. Croat.* 2005; 34, 29-35.
19. Bonifaz, A., Rios-Yuil, J.M., Arenas, R., Araiza, J., Fernández, R., Mercadillo-Pérez, P., Ponce-Olivera, R.M. Comparison of direct microscopy, culture and calcofluor white for the diagnosis of onychomycosis. *Rev. Iberoam. Micol.* 2013; 30, 109-111.
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2007); 1-11.

21. Pemán, J., Martín-Mazuelos, E., Rubio, M.C. In: Linares, M.J., Solís, F. (Ed.). Guía práctica de Identificación y Diagnostico en Micología Clínica, segunda edición (Revista Iberoamericana de Micología 2007): 1-20.

22. Elewski, B.E., Tosti, A. Risk factors and comorbidities for onychomycosis. Clinical Aesthetic 2015; 8, 38-42.

23. Salas-Campos, I., Groos-Martínez, N.T. Agentes etiológicos de onicomicosis diagnosticadas en el laboratorio de micología médica de la Universidad de Costa Rica. Acta Med. Costarric. 2012; 54, 114-118.

24. Méndez-Tovar, L.J., Mejía-Mercado, J.A., Manzano-Gayosso, P., Hernández-Hernández, F., López-Martínez, R., Silva-González, I. Frequency of invasive fungal infections in a Mexican High-Specialty Hospital. Experience of 21 years. Rev. Med. Inst. Mex. Seguro Soc. 2016; 54, 581-587.

25. Sandoval, C., Gutiérrez, F., Fernández, R., Vásquez, E., Moreno, G., Arenas, R. Onicomicosis: incidencia en pacientes con insuficiencia renal crónica y hemodiálisis. Dermatologia CMQ 2014; 12, 172-174.

26. Chan, H.H.L., Wong, E.T., Yeung, C.K. Psychosocial perception of adults with onychomycosis: a blinded, controlled comparison of 1,017 adult Hong Kong resident with or without onychomycosis. BioPsychoSocial Medicine 2014; 8,15.

27. Elewski, B.E. Onychomycosis: pathogenesis, diagnosis, and management. Clin. Microbiol. Rev. 1998; 11, 415-429.

28. Li, M., Chen, Z., Yin, S., Xue, R., Chen, Z., Huang, H., Wei, L., Lu, C., De Hoog, S., Lai, W., Feng, P. Onychomycosis secondary to onychomadesis: an underdiagnosed manifestation. Mycoses 2017; 60, 161-165.

29. Imbert JL, Gomez JVG, Escudero RB, Blasco JL. Onychomycosis by yeast not common in diabetics of a health center. SEMERGEN 2016; 42, 449-457.