Journal of Applied & Environmental Microbiology, 2016, Vol. 4, No. 2, 30-33
Available online at http://pubs.sciepub.com/jaem/4/2/1

DOI:10.12691/jaem-4-2-1

Fungi as Pathogens of Onychomycosis among Diabetic Patients

Saleh. H. Baiu[1,*], Warda M.B. Bridan[2], Hanan. M – K. Kalfa[3]

[1]Department of Botany, Faculty of Science, Benghazi University- Libya
[2]Department of Microbiology, the Libyan Academy, Benghazi- Libya
[3]Department of Dermatology, Jumhuria Hospital, Benghazi – Libya
*Corresponding author: Rose2010dd@gmail.com

Abstract The purpose of the study was to determine the role of dermatophytes, yeasts, and non-dermatophytic moulds as causative agents of onychomycosis among diabetic patients during the months September 2013 to January 2014 in202 diabetic patients suspected to having onychomycosis. The study included each patient from type 2 diabetes mellitus (T2DM) from all patients who were registered at the Sedee Hussein Polyclinic of Benghazi city. The study group equally consisted of 101(50%) male patients and 101(50%) female patients. **Methods:** The specimens were tested by direct microscopic examination using potassium hydroxide(20%) and culturing on Sabouraud's dextrose agar and fungobiotic agar containing cyclohexamide and chloramphenicol. **Results:** The prevalence of onychomycosis among diabetic patients in our study was high (77.2%) in type 2 diabetes mellitus (T2DM). Culture was positive in 156 of 202 diabetic patients with onychomycosis of non-dermatophytic moulds isolated from 91 cases (58%). While *Candida* species have emerged as second-line pathogens, were isolated from fourty one patients (26%). Dermatophytes were detected in only nine patients (6%), and mixed fungi 15 (10%). Distal and lateral subungual onychomycosis was the commonest clinical type (69.2%) followed in decreasing order by total dystrophic onychomycosis (20.5%), and then superficial white onychomycosis (7.7%) and proximal subungual onychomycosis (2.6%). **Conclusion:** This study had confirmed that diabetic patients are at a high risk of having onychomycosis. Managing onychomycosis in diabetic patients may require systemic antifungal treatment, physical measures and patient education.

Keywords: *Non-dermatophytes, dermatophytes, Onychomycosis, Diabetes mellitus*

Cite This Article: Saleh. H. Baiu, Warda M.B. Bridan, and Hanan. M – K. Kalfa, "Fungi as Pathogens of Onychomycosis among Diabetic Patients." *Journal of Applied & Environmental Microbiology*, vol. 4, no. 2 (2016): 30-33. doi: 10.12691/jaem-4-2-1.

1. Introduction

Diabetes mellitus is a worldwide problem of increasing importance. It is well known that diabetic patients often have problems with their feet, due mainly to neuropathy and arterial insufficiency. The risk of toe or lower leg amputation may be increased if fissures or traumatic ulcerations are followed by a secondary bacterial infection [1]. A mycotic nail may be the first step in such a process, as an abnormal nail plate may cause lesions in the surroundings due to hyperkeratosis and sharp nail edges. Fungal nail infection is about four times more common in toenails than fingernails and can involve all or part of the nail, including the nail plate, nail-bed and root of the nail. It is more common in adults than children and more common in men than women. The chances of fungal nail infection increases as we get older [2].

A mycotic infection of the nail unit, is caused by three groups of fungi, namely dermatophytes, yeasts, and non-dermatophytic moulds [2]. In recent years, non dermatophytes have been implicated in probable Nail changes [3].

Although fungal nail infections are not life-threatening, yet they are associated with secondary bacterial infection, therapeutic, failures and disfigurement like hyperkeratosis, discoloration of nail plate, and brittle nails [4]. onychomycosis is classified into distolateral subungual onychomycosis (DLSO), superficial white onychomycosis (SWO), proximal subungual onychomycosis (PSO), candidal onychomycosis (CO) and total dystrophic onychomycosis (TDO) [5]. Non-dermatophytic moulds are filamentous fungi which are commonly found in nature as soil saprophytes and plant pathogens [6]. It is not known whether non-dermatophytic infections occur as a primary ailment on healthy nails, or exist as secondary invaders in already damaged nails by ischemia, trauma or other diseases [3]. Non-dermatophytic moulds (NDMs) which are identified in onychomycosis include *Alternaria* species, regularly *Scytalidium species, Fusarium species, Acremonium* species *Scopulariopsis species, Cladosporium species* and *Aspergillus* species.

The causative agents of the disease may vary depending upon geographic or temporal distribution [3]. Even in developed countries, the importance of nail infections has been highlighted only in the last decade [7].

2. Material and Methods

2.1. Collection of Samples

Two hundred and two T2DM patients with different ages and both sexes were enrolled in the study. Excluding criteria were patients on systemic antifungal agents during 3 month or on topical agents during one month prior to the study. Structured Performa for history, clinical examination and laboratory findings were recorded. The samples were collected from the patients who were suspected to have onychomycosis. They were examined by direct microscopy and cultured at the Sedee Hussein Polyclinic of Benghazi city from September 2013 to January 2014. The specimens were obtained from clinically abnormal nails, by scraping the nail bed, the underside of the nail plate and the hyponychium, after cleaning the affected areas with 70% alcohol (Bode Chemie Hamburg - Germany). For each patient, a separate scalpel blade (Gowllands, England) and a sterile nail clipper (Rshengsl - China) were used for collection of the material to be examined.

The different clinical patterns distal and lateral subungual onychomycosis(DLSO), the proximal subungual onychomycosis(PSO), the white superficial onychomycosis(SWO), and the total dystrophic onchomycosis (TDO) were recorded separately.

Every collected specimen was divided into two parts for the following:

2.2. Derect Microscopic Examination

The collected specimen was placed in a test tube (Assistant, Germany) and fews drops of KOH (20% solution) were added using eye dropper to the glass tube and kept for 24 hours to dissolve the keratin [8]. The collected specimen is then placed on a glass slide (Hamburrg - Germany), and covered by a cover glass (Hamburrg – Germany). Repeated KOH examination was performed before the specimen was considered as negative for direct microscopic mount.

2.3. Cultivation of the Specimens

The specimen of each patient was placed in separate sterile Petri dish. Each specimen was inoculated on sabouraud`s dextrose Agar (SDA), and Fungobiotic agar. The inoculated plates were kept in the incubator (MMM-Grafelfing, Germany) which was adjusted at 28°C and the cultures were examined every two days. The culture was considered negative if there was no growth after four weeks of incubation [9].

The positive specimens (fungi cultures) were mounted with the lactophenol cotton blue to reveal various structures which could be of great help in identification, especially the conidia which include the large separated macroconidia and the small celled microconidia. The macroconidia of each genus and species vary in shape and character of their walls which are generally characteristic for the species or genus [9].

The identification of Candida was based on the presence of budding cells and pseudohyphae.

BD PHOENIX (Becto, Dickinson /USA) is an automated microbiology system intended for the *in vitro* rapid identification (ID)of yeast and yeast like organisms [10].

2.4. Statistical Analysis

Frequency tables and chart constructed for our data were analyzed statistically using the chi-square test. We assumed results statistically significant when P value is < 0.005. The statistical analysis of the results were carried out according to the computer package (SPSS 18.0 version).

3. Results

Out of 202 nail specimens, fungal infection of toenail was found in156 (83% females and 73%) males Age of the patients ranged from 30 to 94 years; mean age = 61.1 years. Seventy two (46.2%) patients were below 60 years and 84 (53.8%) were over 60 years. Fifty-six (35.9%) patients had onychomycosis in the age group of 60- 69 years.

From the 202 cases of suspected onychomycosis 156(77.2%), non- dermatophytic molds and were the most common fungal elements isolated (58%, n = 91) followed by yeast (26%, n = 41),dermatophyte (6%, n = 9),and mixed fungi (10%, n = 15).

Distal and lateral subungual onychomycosis (DLSO) was the commonest clinical pattern (69.2%) followed by total dystrophic onychomycosis (20.5%) then superficial white onychomycosis (7.7%) and proximal subungual onychomycosis (2.6%) (Table 1).

Table 1. Frequency of various clinical types according to sex.

Clinical types	Male	Female	Total	%
DLSO	65	43	108	69. 2
TDO	12	20	32	20.5
WSO	8	4	12	7.7
PSO	2	2	4	2.6
Total	87	69	156	100%

Among the NDMs, *Aspergillus niger* was the most common isolate followed by *Penicillium spp.* And *Cladosporium spp.* Other alsolates were *Aspergillus flavus, Fusarium spp., Syncephalastrume spp., Alternaria spp., Chaetomium spp., Rhizopus spp., Aspergillus glaucus, Scytalidium sp.,Geotrichum candidum, Scopulariopsis sp, Nigrospora sp., Bipolaris sp. and Acremonium sp.* (Table 2).

Table 2. Pattern and frequency of NDMs isolated (n=91)

Species	Total	%
Aspergillus niger.	19	21
Penicillium spp.	14	15
Cladosporium spp.	9	10
Aspergillus fumigatus.	8	9
Aspergillus flavus	6	7
Fusarium spp	6	7
Syncephalastrume spp	5	6
Alternaria spp.	5	6
Chaetomium spp.	4	4
Rhizopus spp.	3	3
Aspergillus glaucuss	2	2
Scytalidium sp	2	2
Geotrichum candidum.	2	2
Scopulariopsis sp.	2	2
Nigrospora sp	2	2
Bipolaris sp.	1	1
Acremonium sp	1	1

Among yeasts, *Trichosporon asahii*. was the most common followed by *Candida parapsilosis, Candida albicans, Trichosporon inkin, Candida tropicalis* and *Candida rugosa*. (Table 3).

Table 3. Pattern and frequency of yeasts isolated (n=41).

Species	Total	%
Trichosporon asahii	20	48.8
Candida parapsilosis complex.	8	19.5
Candida albicans	6	14.6
Trichosporon inkin..	3	7.3
Candida tropicalis.	2	4.9
Candida rugosa.	2	4.9

Among dermatophytes, *Trichophyton mentagrophytes* was the most common isolate followed by *Trichophyton rubrum* and *Trichophyton soudanense.*(Table 4).

Table 4. Pattern and frequency of dermatophytes isolated (n=9)

Species	Total	%
Trichophyton mentagrophytes.	4	44.4
Trichophyton rubrum.	3	33.3
Trichophyton soudanense.	2	22.2

4. Discussion

The prevalence of onychomycosis among diabetics confirmed by culture was77.2% (n=156).In earlier studies the prevalence ranged from of 17 to 30% [11,12,13]. In the present study the prevalence of onychomycosis was nearly equal between males and females. Some studies have reported that onychomycosis more common among males and other studies have found no difference in gender distribution [14,15,16].

In this study, age greater than 50 years was non significantly associated with presence of onychomycosis. Other studies have also reported a higher prevalence of onychomycosis among the elderly [14,15]. The most frequently isolated fungal element in this study was non-dermatophytic moulds (58%, n=91). Similar findings have been reported in Malaysia [13]. Other studies have shown yeasts and dermatophytes as common pathogens isolated from diabetics with onychomycosis [14,17]. This is probably because other factors, such as environment, level of humidity and the repeated contact with water influenced the growth of particular fungi [18]. *Aspergillus spp*. have been found to be the predominating causative molds (21%). In this study non-dermatophytes accounted of the total fungal isolates. Amongst NDMs *Aspergillus niger* was the most common isolate followed by *Penicillium spp*, *Caldosporium spp*., *Aspergillus fumigatus*, *Aspergillus flavus* and *Fusarium spp*. The prevalence of NDMs varied considerably in different studies reported in the literature [9]. In a study conducted in 2006 in Egypt on 32 patients with different nail abnormalities, it was found out that NDMs were isolated from 59% of the total culture positive cases [19]. Comparable results were seen in a local study conducted at Rawalpindi in 2007 which showed that among non-dermatophytes *Alternaria alternate* was the most commonly isolated species followed by *Scytalidium dimidiatum*, and *Penicillium marneffei* [20]. A multi-centre study conducted on a large scale in North America to find out the frequency of pathogens involved in onychomycosis revealed that NDMs and yeasts accounted for 20% each of the two varieties [21]. In a study conducted in Italy revealed *Fusarium* species as the most common NDMs followed by *Scopulariopsis brevicaulis*, *Acremonium* and *Aspergillus* species [6]. Studies carried out in Sri Lanka, [22], Colombia [23], and Pakistan [20], revealed large percentage of *Fusarium spp*. from patients of onychomycosis. On the contrary, the most common NDM isolated in studies reported from Europe [24]. North America [6] and Mexico [25]. were *Scopulariopsis* followed by *Aspergillus.*

However, in one Indian study, yeasts were found to be the most common pathogens causing onychomycosis in diabetic patients, followed by dermatophytes and non-dermatophytic moulds [26].

Another study from Saudi Arabia reported that *Candida* species were the most frequently isolated pathogen from infected nails [27]. In Kuwait, dermatophytes were the most common isolates causing onychomycosis in diabetic patients, followed by yeasts and non-dermatophytic moulds [26].

DLSO was the commonest clinical presentation in this study(69.2%). which is comparable with most of the Previous studies. [28,29,30].

Thus, the climates and geographical locations may influence the distribution of the pathogens of onychomycosis as well. The major pathogens in temperate western countries are dermatophytes, but *Candida* and nondermatophytic moulds are the prevailing pathogens in mediterranean and tropical countries with a warmer and more humid climate. Regional variance in the distribution of pathogens may be partially ascribed to the occupations of residents.

Onychomycosis in diabetics may be associated with diabetic foot ulcers, cellulitis and gangrene. These complications increase rates of hospital admissions and surgical interventions. Early diagnosis and adequate medical management of onychomycosis among diabetics using oral antifungal agents may be more cost effective than treating the complications arising from delayed diagnosis and treatment [14].

5. Conclusion

This study shows a high prevalence of onychomycosis among diabetics. Onychomycosis is associated with a higher risk of complications among diabetics, Physicians should actively examine the feet for onychomycosis during routine consultation.

References

[1] Rich, P. Onychomycosis and tinea pedis in patients with diabetes. *J Am Acad Dermatol*, (2000), 43: 130-134.

[2] Summerbell, R.C., Kane, J., Krajden SOnychomycosis, tinea pedis and tinea manuum caused by non-dermatophytic filamentous fungi. *Mycoses*; (1989), 32:609-19.

[3] Agarwalla, A., Agrawal, S., Khanal, B., Onychomycosis in eastern Nepal. *Nepal Med Coll J*, (2006), 8:1-7.

[4] Jarve, H., Naaber, P., Kaur, SToe nail onychomycosis in Estonia. *Mycosis* 2004, 47:57-61.

[5] Scher PK. Onychomycosis: a significant medical disorder. *J Amer Acad Dermato* (1996); 35: S2-S5

[6] Tosti, A., Piraccini, B.M., Lorenzi, SOnychomycosis caused by non-dermatophytic molds. *J Am Acad Dermatol*; (2000). 42: 217-24.

[7] Weeks, J., Elewski, B., Management of superficial infections. In: Merz WG, Hay RJ, editors. Topley and Wilson's microbiology and microbial infections. 10th ed. London: *Hodder Arnold*, (2005). p. 182-9.

[8] Lim, J. T.; Chua, H. C. and Goh, C. L. Dermatophyte and non dermatophyte onychomycosis in Singapore. *Australas. J. Dermatol*; (1992). 33: 159-163.

[9] Ramani, R.; Srinivas, C. R.; Ramani, A.; Kumari, T. G. and Shivananda, P. G. Molds in onychomycosis, *Int. J. Dermatol*; (1993).32: 877-878.

[10] Kampfer, P.; Rauhof, F. O.; and Dott, W. (1991). Glycosidase Profiles of Members of the Family *Enterobacteriaceae*, *J. Clin. Microbiol.* 29:2877-2879.

[11] Dogra, S., Kumar, B., Bhansali, A., Chakrabarty, A., Epidemiology of onychomycosis in patients with diabetes mellitus in India. *Int. J. Dermatol*, (2002), 41: 647-51.

[12] Chang, S. J., Hsu, S. C, Tien, K. J., Hsiao, J. Y., Lin, S. R. and Chen, H. C. Metabolic syndrome associated with toenail onychomycosis in Taiwanese with diabetes mellitus. *Int. J. Dermatol*, (2008), 47: 467-72.

[13] Leelavathi, M.; Azimah, M. N.; Kharuddin, N. F.; Tzar, M. N.. Prevalence of toenail onychomycosis among diabetics at a primary care facility in Malaysia. *J. Trop. Med. Public. Health*, (2013), 44(3):479-483.

[14] Gupta, A. K., Konnikov, N., MacDonald, P., Rich, P.; Rodger, N. W., Edmond s, M. W.; and *et al*. Prevalence and epidemiology of toenail onychomycosis in diabetic subjects: a multicentre survey. *Br. J.Dermato*, (1998), 139:665-71.

[15] Saunte, D. M., Holgersen, J.B., Haedersdal, M.; Strauss, G. and Bitsch, M. Svendsen OL. Prevalence of toenail onychomycosis in diabetic patients, *Acta Derm Venereol*; (2006), 86: 425-8.

[16] Kafaie, P.; Noorbala, M. T. Evaluation of onychomycosis among diabetic patients of Yazd diabetic centre. *J. Pakistan. Assoc Dermatol*,, (2010), 20: 217-21.

[17] Manzano-Gayosso, P., Hernández-Hernández, F., Méndez-Tovar, L. J., Palacios-Morales, Y., Córdova-Martínez, E.; Bazán –[Mora, E., Onychomycosis incidence in type 2 diabetes mellitus patients. Mycopathologia; (2008)., 166: 41-5.

[18] Berker, D. Clinical practice. Fungal nail disease, *N. Engl. J.Med*,, (2009), 360: 2108-16.

[19] El Batawi MM, Arnaot H, Shoeib S, Bosseila M, El Fangary M, Helmy AS. Prevalence of non-dermatophyte molds in patients with abnormal nails. *Egyptian Dermatol Online J*(2006); 2:11

[20] Hanif, F., Ikram, A.; Butt, T., Malik, N., Qadir, I.H., Faiz, UTrends of fungal isolates in our set up. *Infect Dis J Pak*.(2009)., 18:3-5.

[21] Ghannoum MA, Hajjeh RA, Scher R, Konnikov N, Gupta AK, Summerbell R, *et al.* A large scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and anti-fungal susceptibility patterns. J Am Acad Dermatol(2000); 43:641-8.

[22] Ranawaka, R.R., De Silva, N., Ragunathan, R.W Onychomycosis caused by *Fusarium* spp. in Sri Lanka: prevalence, clinical features and response to itraconazole pulse therapy in six cases. *J Dermatolog Treat*.(2008).; 19:308-12.

[23] Castro, L.N., Casas, C, Sopo, L.;Rojas, A., Del Portillo, P.; Cepero MC, *et al*.,*Fusarium* species detected in onychomycosis in Colombia. *Mycoses*; (2008). 23:121-4.

[24] English MP. Comment. Nails and fungi. *Brit J Dermatol* 1998; 94: 481-90.

[25] Bonifaz A, Angular CP, Ponce RM. Onychomycosis by molds: report of 78 cases. *Eur J Dermatol*(2007); 17:70-2.

[26] Dogra, S.; Kumar, B.; Bhansali, A. and Chakrabarty, A. (2002). Epidemiology of onychomycosis in patients with diabetes mellitus in India. *Int. J. Dermatol*; 41: 647-51.

[27] Sogair, S. M.; Moawad, M. K. and Al-Humadan,Y. M. (1991). Fungal infection as a cause of skin disease in the eastern province of Saudi Arabia: prevailing fungi and pattern of infection. *Mycoses*;34:333-7.

[28] Baran R, Hay RJ, Tosti A, Haneke R. A new classification of onychomycosis. Br J Dermatol(1998);139:567-71.

[29] Faergemann J, Baran R. Epidemiology, clinical presentation and diagnosis of onychomycosis; BrJDermatol(2003),149. (Suppl 65):1-4.

[30] Romano C, Gianni C, Difonzo EM. Retrospective study of onychomycosis in Italy: 1985-2000.Mycose(2005);48:42-4.