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ONYCHOMYCOSIS IN WORKERS FROM A COMMUNITY GARDEN IN TERESINA, PIAUÍ, BRAZIL

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ABSTRACT

Onychomycosis is an infection of the nail blade caused by dermatophytes, yeasts and non-dermatophyte fungi. This study aimed to determine the prevalence of onychomycosis in horticulturists of Piauí, identifying the causative fungal etiological agents. A total of 125 horticulturists clinically suspected of onychomycosis were examined for causative fungal agents. In this sense, three subungual samples were collected from each individual, followed by direct KOH examination and subsequent *in vitro* culture. The fungal species were identified by means of morphological, biochemical and molecular analyses. Sixty six out of the 125 horticulturists (52.8%) had confirmed clinical onychomycosis, of which 73.3% had 50 years or more; 33.6% had same risk factors; 66.7% had diabetes and 94% didn't use any Personal Protective Equipment (PPE). Sixty nine fungal isolates were identified as the etiological agents as follows: 52 non-dermatophyte, 16 yeasts and only 1 dermatophyte. In this study, horticulturists with older age and that didn't use PPE were the more susceptible to develop this disease. Our results indicate, for the first time, a frequency above 70% of non-dermatophyte fungus agent of onychomycosis.

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INTRODUCTION

Onychomycosis is a chronic infection of the nail blade caused by dermatophytes, not dermatophytes and/or yeast. It is currently an important problem of public health because it affects about 5% to 10% of the worldwide population, representing more than 50% of all nail pathology (Allevato, 2010; Thomas et al., 2010; Zotti et al., 2011; Zalacain et al., 2011; Soltani et al., 2015). The onychomycosis presents big therapeutic difficulties related mainly with advanced age, poor drug penetration into the nail and with a late or inaccurate diagnosis (Zalacain et al., 2011; Mikaeili and Karimi, 2013).

Among the preponderant factors for development of this disease, the immunosuppression, psoriasis, lifestyle, practice of sports and occupational factors really stand out (Schechtman, 2008; Ranawaka et al., 2012). According to existing literature, the most commonly isolated fungi from patients with onychomycosis belong to the group of dermatophytes and yeasts, being *Trichophyton* spp. and *Candida* spp. the main representatives, respectively. Onychomycosis caused by non-dermatophyte fungi (NDF) are not very much reported, being *Scopulariopsis* spp., *Aspergillus* spp. and *Fusarium* spp. the NDF more associated with nail mycoses (Tosti et al., 2000; Bonifaz et al., 2013; Nouripour-Sisakht et al., 2015). In the Brazil there are few studies that discuss the onychomycosis, most of them is distributed in the industrially developed Brazilian States like São Paulo and Rio

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de Janeiro. In Teresina, the capital city of the state of Piauí, northeast area of the country, until the moment there isn't any reference for this disease. Facing this reality, this study aimed to determine the prevalence of onychomycosis in horticulturist of Piauí and to identify the causative fungal agents.

MATERIALS AND METHODS

Population

This study was reviewed and approved by the Centro Universitário UNINOVAFAPÍ Ethics Committee, with CAAE 02070043000-10. It was considered able to take part of this study all the horticulturists who worked at least 12 months and presented nail changes, such as color change and/or full or partial deformity. The workers that refused to sign the Enlightened Free Consent Term (EFCT) were not considered able for this research. In order to be accepted in the study, it was applied a structured questionnaire comprising demographic data (age and genre), period of activity in the garden, pre-existing disease(s) and use of Personal Protective Equipment (PPE) while working.

Collect

The samples were collected from the nails of the hands or feet of the horticulturists in dry and rainy seasons. The workers were oriented not to use enamel or topical medications for, at least, two weeks. Before the collection, the nails were washed with a brush, soap and water. Immediately after washing it was performed the antisepsis of the affected nails with 70% alcohol. Three samples of subungual debris were collected from each volunteer, with 7 to 10 day intervals, with the aid of a disposable scalpel blade. The samples were then sent to the Research Laboratory of the UNINOVAFAPÍ.

Analysis

Each nail sample was divided into two portions: one for direct exam with potassium hydroxide (KOH 20%) and the other to culture in tubes containing Sabouraud dextrose agar (Difco, DetroitTM, MI, France) with Chloramphenicol (INLAB, São Paulo, Brazil). Three tubes were used for each sample. All the tubes were incubated at room temperature (30 °C ± 2 °C) for a period of 7 to 15 days or until the development of yeast or filamentous colonies. *Only the colonies of NDF that grew in 2 of the 3 tubes seeded and obtained in the 3 collections were selected, being this the criterion used to consider NDF as the etiologic agent of the disease (Mikaeili and Karimi, 2013). The identification keys described by De Hoog et al. (2000) and Lacaz et al. (2002) were used for phenotypic identification. As definitive test of identification, was used PCR with specific primers with starting sequences indicated in literature (White et al., 1990).*

Statistical analysis

In the analysis of the data, the binomial test was used for independent samples with significance level (α) of 5% to observe the differences between proportions. The Fisher's exact test with α of 5% was applied in the analysis of the existence of association between the identified species and the age group of the horticulturists. The questionnaires answered by horticulturists were processed in the Program IBM SPSS

Statistics 20.0 e 21.0 (Statistical Package for the Social Sciences). Values of $P \leq 0.05$ were considered significant.

RESULTS

Altogether, 125 horticulturist were included into the study (68 female, 57 male). Of these, sixty six had clinical onychomycosis confirmed by isolation of the same agent in three samples collected in sequence. Out of the 66 workers with onychomycosis, 54.4% (33/68) corresponds to the sex feminine and 45.6% (33/57) to the sex masculine. There was no significant association between gender and presence of onychomycosis ($P = 0.30$). We identify 69 etiologic agents of onychomycosis: 52 non-dermatophyte, 16 yeasts and only 1 dermatophyte. As for distribution of the lesions, 53% of them were on the toenails and 47% on the fingernails. The table I summarizes the distribution and frequency of the isolated fungi.

The age group with larger number of fungal isolates was that of 50 years or more, followed by the 40 to 50 years old. *Aspergillus clavato-nanicus* was found only in age group of 20-30 years old, being more frequently age group from 50 years or more. The relation between species and age group is expressed in table II. It was observed that 33.6% of the horticulturists have risk factors for the development of onychomycosis and in 50.4% from workers this information was not obtained. The table III show that nine (13.6%) out of the 66 positive horticulturists to this disease also confirmed possessing one or more basic disease for this onychopathy. The Diabetes, with 66.7%, was the most prevalent disease among those diagnosed with onychomycosis, followed by the association of diabetes and hypertension (22.2%). The binomial test no showed correlation between the underlying diseases and the presence of onychomycosis ($P=1.0$). In relation to the use of Personal Protective Equipment (PPE), we observed that 94% of the diagnosed individuals with onychomycosis didn't use any PPE and only 6% used boots during the work performed in the garden.

DISCUSSION

The nails, as components of the fingertips, are structure integrant of the sensory part of the hand. The loss of the free margin of the nail for onychomycosis can reduce the sensory capacity of the fingers with a consequent limitation of manual dexterity. On the other hand, this disease in the feet can cause pain and discomfort, making it difficult to walk and practice sports, resulting in a significant damage to health in general. This onychopathy also causes important psychological consequences, as for example anxiety, worries with appearance, depression and embarrassment in intimate situations (Peres et al., 2010; Gunduz et al., 2014). Non-dermatophyte filamentous fungi (NDF) comprise a broad, heterogeneous and cosmopolitan group that inhabit vegetables and soils. Together with yeasts, the NDF are more frequently described in countries with hot and humid climates, while the dermatophytes are more isolated in temperate climates (Chi et al., 2005; Martelozo et al., 2005). These facts may explain the higher prevalence of NDF and yeast as agents of onychomycosis in this research, since Teresina is the city with the highest temperatures and humidity rates of the country and the population studied has intimate contact with the habitat of these fungi. In Brazil, few studies accurately describe NDF as onychomycosis agents (Araújo et al., 2003; Martelozo et al., 2005; Oliveira et al., 2006; Martins et al., 2007).

Table 1. Distribution and frequency of the isolated fungi

Fungal pathogens	Location of lesion					
	Fingernails		Toenails		Total	
	N°	%*	N°	%*	N°	%*
Non-dermatophyte						
<i>Aspergillus clavato-nanicus</i>	6	19,4	10	28,6	16	24,2
<i>Neoscytalidium hyalinum</i>	6	19,4	4	11,4	10	15,2
<i>Acremonium hyalinulum</i>	3	9,7	3	8,6	6	9,1
<i>Neoscytalidium dimidiatum</i>	2	6,5	3	8,6	5	7,6
<i>Paecilomyces variotii</i>	1	3,2	2	5,7	3	4,5
<i>Bipolaris cf. hawaiiensis</i>	1	3,2	1	2,9	2	3,0
<i>Curvularia lunata</i>	1	3,2	1	2,9	2	3,0
<i>Fusarium oxysporum</i>	2	6,5	-	-	2	3,0
<i>Rhinochadiella aquaspersa</i>	-	-	2	5,7	2	3,0
<i>Aspergillus flavus</i>	-	-	1	2,9	1	1,5
<i>Fusarium solani</i>	-	-	1	2,9	1	1,5
<i>Nigrospora aff. sphaerica</i>	-	-	1	2,9	1	1,5
<i>Ramichloridium aff. Schulzeri</i>	-	-	1	2,9	1	1,5
Yeasts						
<i>Candida albicans</i>	3	9,7	1	2,9	4	6,1
<i>Candida parapsilosis</i>	2	6,5	1	2,9	3	4,5
<i>Candida guilliermondii</i>	-	-	2	5,7	2	3,0
<i>Candida metapsilosis</i>	1	3,2	1	2,9	2	3,0
<i>Trichosporon asahii</i>	1	3,2	1	2,9	2	3,0
<i>Candida africana</i>	1	3,2	-	-	1	1,5
<i>Candida krusei</i>	1	3,2	-	-	1	1,5
<i>Candida rugosa</i>	1	3,2	-	-	1	1,5
Dermatophyte						
<i>Microsporum nanum</i>	1	3,2	-	-	1	1,5
Total	33	100	36	100	69	100

Source: Research Laboratory of the UNINOVAFAPI.

*Sum more than 100% may be more than one species of fungus in a single worker.

Table 2. Fungal isolates according with age group of horticulturists.

	Age group								p
	20 ---30		30 ----40		40 ----50		50 or +		
	N°	%*	N	%*	N°	%*	N°	%*	
Non-dermatophyte									
<i>Aspergillus clavato-nanicus</i>	1	100	-	-	2	15,4	13	26,5	0,2
<i>Neoscytalidium hyalinum</i>	-	-	-	-	2	15,4	8	16,3	0,3
<i>Acremonium hyalinulum</i>	-	-	1	33,3	1	7,7	4	8,2	0,3
<i>Neoscytalidium dimidiatum</i>	-	-	-	-	2	15,4	3	6,1	0,3
<i>Paecilomyces variotii</i>	-	-	1	33,3	-	-	2	4,1	0,4
<i>Bipolaris cf. hawaiiensis</i>	-	-	-	-	-	-	2	4,1	0,5
<i>Curvularia lunata</i>	-	-	-	-	1	7,7	1	2,0	0,4
<i>Fusarium oxysporum</i>	-	-	-	-	1	7,7	1	4,1	0,7
<i>Rhinochadiella aquaspersa</i>	-	-	-	-	-	-	2	4,1	0,5
<i>Aspergillus flavus</i>	-	-	1	33,3	-	-	-	-	0,3
<i>Fusarium solani</i>	-	-	-	-	-	-	1	2,0	0,4
<i>Nigrospora aff. sphaerica</i>	-	-	-	-	-	-	1	2,0	0,7
<i>Ramichloridium aff. Schulzeri</i>	-	-	-	-	1	7,7	-	-	0,3
Yeasts									
<i>Candida albicans</i>	-	-	-	-	-	-	4	8,2	0,3
<i>Candida parapsilosis</i>	-	-	-	-	1	7,7	2	4,1	0,4
<i>Candida guilliermondii</i>	-	-	-	-	1	7,7	1	2,0	0,4
<i>Candida metapsilosis</i>	-	-	-	-	1	7,7	1	2,0	0,4
<i>Trichosporon asahii</i>	-	-	-	-	-	-	2	4,1	0,5
<i>Candida africana</i>	-	-	-	-	-	-	1	2,0	0,7
<i>Candida krusei</i>	-	-	-	-	-	-	1	2,0	0,7
<i>Candida rugosa</i>	-	-	-	-	-	-	1	2,0	0,7
Dermatophyte									
<i>Microsporum nanum</i>	-	-	-	-	-	-	1	2,0	0,7
Total	1	100	3	100	13	100	52	100	-

Table III. Base disease and occurrence of onychomycosis in horticulturists

Base disease	Onychomycosis					
	Yes		Not		Total	
	N°	%	N°	%	N°	%
Diabetes	6	66,7	3	60,0	9	64,3
Chronic renal failure	1	11,1	-	-	1	7,4
Diabetes and hypertension	2	22,2	1	20,0	3	21,4
Hypertension and chronic renal failure	-	-	1	20,0	1	7,4
Total	9	100	5	100,0	14	100

Source: Research laboratory of UNINOVAFAPI

Even in these few reports, the frequencies of this group of fungi were much lower (7.9-39%) than the verified in this study (78.3%). According Galimbert and collaborators (2012), *Aspergillus* spp. is the third or fourth NDF more reported as onychomycosis agent. The species of this genus most frequently isolated from this onychopathy are: *A. flavus*, *A. versicolor*, *A. terreus*, *A. niger*, *A. fumigatus* *A. sydowii* (Araújo et al., 2003). *Aspergillus clavato-nanicus*, species most frequent in this study, was cited as onychomycosis agent first by Batista and colleagues (1955), after analysis samples collected from fingernail of the left hand of a washerwoman in Recife – PE, Brazil. In relation to the distribution of fungal isolates, onychomycosis in toenails was the most frequent in the evaluated population. Statistically, in this research there was no correlation between the species and the site of the lesion, although the literature indicates that *Aspergillus* spp. is more prevalent in foot injuries.

Regarding gender, studies conducted in Canada indicated the masculine genre as the most frequent for onychomycosis (Gupta et al., 2000), already in other regions such as Pakistan (Ahmed et al., 2013). Mexico (Bonifaz et al., 2013) and Brazil the feminine gender is more affected by this disease (Martelozo et al., 2005; Araújo et al., 2003; Lima et al., 2009). In this study, even the number of women was superior to men, there were none significant differences between the number of men and women with onychomycosis ($P=0.30$). In relation to age, the majority of horticulturists (74%) who had onychomycosis belonged to the age group of 50 years or more. This finding is in consonance with the literature, since nail diseases are common problems in the geriatric population and may be associated with the peripheral circulation or altered biological mechanism (Gunduz et al., 2014). Is important to note that the *Aspergillus clavato-nanicus* was only fungi found in the age group of 20-30 years old, although their frequency has been higher in the group of 50 years or more (26.5%). However, observing the table II, it can be observed that no species had a significant relation with an age group, since there was no $P \leq 0.05$.

Predisposing factors for onychomycosis include genetic factors, family history, smoking, frequent nail trauma, immunocompromised individuals and sport activities, beyond the factors related to clinical status of the patient, as: renal failure, hypertension and diabetes (Thomas et al., 2010). According to Table III, 40.9% of the horticulturist diagnosed with onychomycosis had some predisposing factor, while what 24.1% affirmed not having any predisposition. However, it should be noted that in 43.9% of the horticulturists it was not possible to get this information, suggesting that the number of patients with this disease and one predisposing factor may be much higher than 40.9%. A study of onychomycosis performed by Ranawaka et al. (2012) with workers in constant contact with soil and water, indicated that it was rare the habit of wearing rubber boots or closed shoes due to hot weather and humidity. The association with illiteracy and low income can also be contributor factors to the low adherence to PPE. In this study, was observed que nine (13.6%), out of the 66 positive workers for onychomycosis, had some base disease and that 94% of horticulturists with nail mycoses did not use PPE. These results emphasize that, although the presence of a base disease is preponderant for the development of onychomycosis, the non-use of PPE, that is, the non-compliance with biosafety standards is significantly more important to the development of onychopathy. In conclusion,

horticulturists with older age and that didn't use PPE were the more susceptible to develop onychomycosis in this study. Factors such as gender and prevalence of base diseases were not significant for the development of this onychopathy in the analyzed population. From an epidemiological point of view, this work is, actually, the first reference in onychomycosis in Piauí, Brazil.

Conflicts of Interests: The authors declare no conflict of interest

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