

# Frequency of mixed onychomycosis with total nail dystrophy in patients attended in a Guatemalan Dermatology Center

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## Abstract

**Introduction:** Onychomycosis are fungal nail infections that can be caused by dermatophytes, non-dermatophytic molds and yeasts, which are capable of breaking down keratin. Mixed onychomycosis are a controversial subject and they are the outcome of the combination of two dermatophytes, dermatophytes/non-dermatophytic molds or dermatophytes/yeast.

**Objetives:** To determine the frequency of total dystrophic onychomycosis caused by more than one etiological agent (mixed onychomycosis) in outpatients from a Dermatologic Center in Guatemala and to establish the characteristics associated with this fungal infection.

**Methods:** Prospective observational study from August to December of 2012. Nail samples were obtained from patients with total dystrophic onychomycosis to identify the causal agents by culture in Sabouraud dextrose and Mycosel® agar.

**Results:** 32 of 130 patients had mixed onychomycosis. 68.5% were associated to *tinea pedis*. The most common association was between *T. rubrum* + *Candida*, *T. rubrum* + *M. canis* and *T. rubrum* + opportunist fungi.

**Conclusions:** Mixed onychomycosis represent 25% of the total dystrophic onychomycosis in Guatemala. We observed an important relationship between diabetes and the main association was *T. rubrum* with *Candida* spp.

**Keywords:** Mixed onychomycosis, dermatophytes, opportunistic fungi

## Frecuencia de onicomiosis mixta con distrofia ungueal total en pacientes atendidos en un Centro de Dermatología de Guatemala

### Resumen

**Introducción:** Las onicomiosis son infecciones fúngicas de las uñas que pueden ser causadas por dermatofitos, mohos no dermatofitos y levaduras, que son capaces de degradar la queratina. Las onicomiosis mixtas son un tema polémico y es el resultado de la combinación de dos dermatofitos, dermatofitos / mohos no dermatofitos o dermatofitos / levadura.

**Objetivos:** Determinar la frecuencia de la onicomiosis distrófica total causada por más de un agente etiológico (onicomiosis mixta) en pacientes ambulatorios de un Centro Dermatológico en Guatemala y establecer las características asociadas a esta infección fúngica.

**Métodos:** Estudio observacional prospectivo de agosto a diciembre de 2012. Se obtuvieron muestras de uñas de pacientes con onicomiosis distrófica total para identificar los agentes causales en cultivo de agar dextrosa Sabouraud y Mycosel®.

**Resultados:** 32 de 130 pacientes tenían onicomiosis mixta. 68.5% se asociaron a *tinea pedis*. La asociación más común fue entre *T. rubrum* + *Candida*, *T. rubrum* + *M. canis* y *T. rubrum* + hongos oportunistas.

**Conclusiones:** La onicomiosis mixta representa el 25% de la onicomiosis distrófica total en Guatemala. Observamos una relación importante entre la diabetes y la asociación principal fue *T. rubrum* con *Candida* spp.

**Palabras clave:** Onicomiosis mixta, dermatofitos, hongos oportunistas

## Introduction

Onychomycosis or *tinea unguium* are fungal infections of the nail<sup>1,2,3,4</sup> and can be caused by dermatophytes, non-dermatophytic molds and yeasts. Usually they carry the necessary enzymes to break down keratin, although, according to the

International Society for Human and Animal Mycology (ISHAM), this term doesn't take into consideration the cause of the infection and the term *tinea unguium* is reserved only for those caused by dermatophytes<sup>4</sup>. Onychomycosis represents up to 50% of nail disorders<sup>1,2,3,4</sup>, affecting 2-18% of the population<sup>5,6,7</sup>, with an incidence of 40-48% among patients

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older than 70 years<sup>4,5</sup>. Many risk factors for onychomycosis have been identified, including age, occupation, occlusive footwear (boots/tennis), diabetes mellitus, peripheral vascular disease, trauma, and *tinea pedis* among others<sup>8,9,10</sup>. Onychomycosis caused by non-dermatophytic molds are more common in HIV patients<sup>11</sup>. The risk of onychomycosis in diabetic patients is 1.9 to 2.8 times higher than the rest of the population<sup>12</sup>. Onychomycosis can be classified according to their morphology and grade of invasion. It has been suggested a classification for onychomycosis that divides them in different clinical patterns: distal and lateral subungual onychomycosis (DLSO), superficial white onychomycosis (SWO), proximal white subungual onychomycosis (PWSO), total dystrophic onychomycosis (TDO), mixed onychomycosis and endonyx onychomycosis<sup>2,13,14</sup>.

Onychomycosis differential diagnosis include chronic paronychia, lichen planus, psoriasis, and melanoma among others<sup>15</sup>. Usually the diagnosis is implemented in two parts, initially a sample taken by scrapping nail debris near the infected area is analyzed through KOH test that in some cases can be enhanced with DMSO (dimethylsulfoxide); the second part can be done through a diverse number of culture mediums, the most commonly used are Sabouraud agar for the isolation of some molds, dermatophytes and yeasts and Mycosel® agar (that contains chloramphenicol and cycloheximide). Onychomycosis diagnosis can also be performed by nail biopsy stained with PAS (Periodic acid-Schiff). When the infection is caused by more than one etiological agent it is known as mixed onychomycosis and it is usually associated with two dermatophytes or dermatophytes/opportunistic mold (that includes yeasts and non-dermatophytic molds). Worldwide prevalence ranges from 11% up to 22%<sup>17</sup> and it has been correlated to therapeutic failure, although this is controversial<sup>1</sup>. The medications that are usually used to treat onychomycosis are amorolfine 5%, ciclopirox 8%, fluconazole, itraconazole and terbinafine, non-pharmaceutical treatments like lasers and photodynamic therapy are being employed too<sup>14</sup>. Therapeutic failure has been linked to immunosuppression, incorrect use of antimycotic agents, short duration of treatments, among others and recurrence can be observed in 10 to 53% of the patients<sup>14,18,19</sup>.

Our aim, in present work, was to determine the frequency of total dystrophic onychomycosis caused by more than one etiological agent (mixed onychomycosis) in outpatients from a Dermatologic Center in Guatemala and to establish the characteristics associated.

## Material and Methods

A prospective observational study was performed in all patients with the clinical diagnosis of TDO (total dystrophic onychomycosis with more than 50% of nail affection and presence of hyperkeratosis) from August to December of 2012, with no age restrictions, no gender restrictions, with no prior or current use of topical or systemic antifungal therapy and

prior approval of informed consent. Patients that had received previous systemic or topical antifungal treatment and that didn't signed or approved the informed consent were excluded. The nail samples were taken by scraping off the distal area of the nail and perforating the proximal area with a No.15 scalpel while avoiding the nail bed. The samples were directly observed using 40% of KOH with DMSO and cultured separately in Sabouraud and Mycosel® agar (supplemented with cycloheximide and chloramphenicol), which were incubated at 25°C for 14 to 25 days. When a non-dermatophytic molds or yeasts was isolated, two more samples were taken, with a 15 day interval between them, cultures were considered positive for non-dermatophytic molds or yeast if they were isolated in two out of the three cultures. The isolated materials were identified through the morphological characteristics of their colonies, as well as the microscopy observed through staining with lactophenol cotton blue solution. To analyze the relationship between comorbidities and total dystrophy onychomycosis a 95% confidence interval was used.

## Results

A total of 130 patients were diagnosed with onychomycosis, 32 (24.61%) of them displayed mixed onychomycosis. The majority of patients affected were females (n=89/130, 68%), of whom 24.71% (n=22/89) had mixed onychomycosis, followed by the males (n=41/130, 32%) of which 24.39% (n=10/41) presented mixed onychomycosis. This infections predominated for both sexes between the ages of 41-60 years old (n=19/32, 59.5%) (table 1). The time of evolution associated with mixed onychomycosis was over 10 years (n = 11/32, 34.4%). Regarding comorbidities, 22 out of 130 patients had diabetes mellitus (17%) which was more frequent in females (n=20/22, 91%) and 16 out of these 22 patients displayed mixed onychomycosis (73% of the total cases of diabetes and 50% of the total cases with mixed onychomycosis), dermatophytic infections were also comorbidities found in the patients (n=62/130, 48%) with *tinea pedis* being the main concomitant one (n=42/62, 68%). 25% of the patients (n=32/130,) referred nail trauma; according to the 95% confidence interval, the most related comorbidities with total dystrophy onychomycosis were the cutaneous dermatophytic infections, (table 2 and 3, graph 1). Fungal agents responsible for mixed onychomycosis were mainly *Trichophyton rubrum*, *Candida* spp., *Microsporum canis*, *Scopulariopsis* spp., *Aspergillus* spp. y *Fusarium* spp. (graph 2). In the case of diabetic

**Table 1.** Age distribution of patients with mixed onychomycosis

Age groups	Female		Male		Total
	Patients/Percentage		Patients/Percentage		
	N°	%	N°	%	
0 – 20	0	0	1	10%	3.1%
21 – 40	1	4.6%	0	0	3.1%
41 – 60	13	59%	6	60%	59.4%
> 61	8	36.4%	3	30%	34.4%
<b>Total</b>	22	68/100%	10	32/100%	100%

**Table 2.** Statistical analysis of risk factors (Comorbidities)

Risk factor	Onychomycosis with total nail dystrophy	Percentage	C.I. 95% Lower limit	C.I. 95% Upper limit
Diabetes Mellitus	130	100%		
Present	22	17%	8.70	25.30
Not present	108	83%	81.00	85.00
Cutaneous dermatophytic infections	130	100%		
associated	62	48%	42.77	53.23
Not associated	68	52%	47.23	56.77
Previous Trauma	130	100%		
Previous	32	25%	24.46	39.54
No previous	98	75%	95.50	100

**Table 3.** Mixed onychomycosis with other dermatophytic infections

Associated cutaneous dermatophytic infections	Number of patients	Percentage
<i>Tinea pedis</i>	13	68.4%
<i>Tinea pedis</i> / onychomycosis	2	10.5%
Fingernails onychomycosis	1	5.3%
<i>Tinea cruris</i>	1	5.3%
<i>Tinea pedis</i> / <i>tinea corporis</i>	1	5.3%
<i>Tinea pedis</i> / <i>tinea cruris</i>	1	5.3%
Total	19	100%

patients, a greater association of dermatophyte *T. rubrum* + *Candida* spp. (n = 10/16, 62.5%) was observed, overall, the greatest association in the cases of mixed onychomycosis was *T. rubrum* + *Candida* spp. (n = 14/32, 44%) (table 4).

**Discussion**

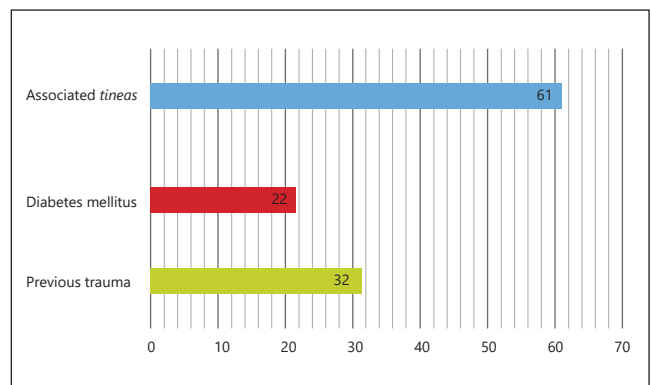
Differences regarding gender and age had been found according to the findings published by Ching et al.,<sup>20</sup> and Wang et al.<sup>21</sup> They reported a higher incidence in men, this was attributed to occupational factors such as the use of close-toed shoes or repetitive local trauma<sup>22</sup>, however, Avelar et al.<sup>23</sup> in Brazil reported a higher incidence in women, stating that they consult the dermatologist more often; on the other hand, Roberts<sup>24</sup> showed no difference between genders. Regarding age, Gupta et al.<sup>25,26</sup>, Hay<sup>27</sup> and Avelar et al.<sup>23</sup>, listed separately a correlation between an increased prevalence of onychomycosis after 55 to 60 years of age, this contrasts slightly with our results, because most of our patients were 41 or beyond; finally, Reddy et al.<sup>28</sup>. reported a higher frequency in the group of 20 to 40 years. Our patients were mostly between 40 to 60 years old.<sup>29</sup>

Sixteen patients (50%) had diabetes, contrasting with those reported by Arenas et al.<sup>30</sup> in Mexico, Gupta et al.<sup>31</sup> in Canada,

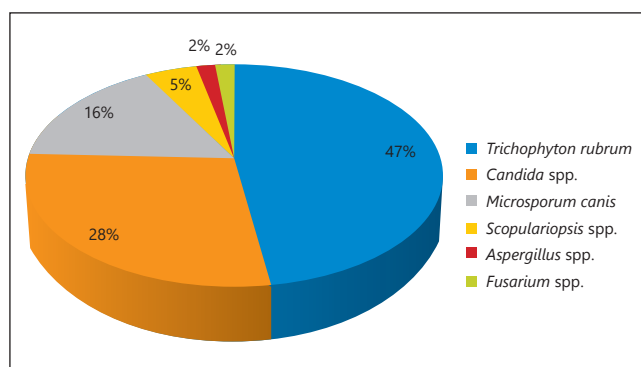
Isa y Arenas<sup>32</sup> in Dominican Republic and Dogra et al.<sup>33</sup> in India, who found an association of 31.5%, 26%, 25% and 17%, respectively. This authors agree that diabetics may have a higher risk, which was demonstrated by Mayser et al.<sup>12</sup>, who showed that the risk is 1.9 to 2.8 times higher, although the literature provides different data<sup>12,34</sup>. There are reports regarding the correlation between glucose and the percentage of fungal infections, all the patients (100%) with serum glucose >300 mg/dl have a fungal disease<sup>35</sup>. About 68.5% of patients with onychomycosis had concomitant *tinea pedis*, this is consistent with other studies, it has been reported that the most often dermatophytosis related to onychomycosis is *tinea pedis*, considering that usually the infection spreads to the nail from the foot<sup>36,37</sup>. Scher y Baran<sup>1</sup>, Ballesté et al.<sup>38</sup>, and Szepietowski<sup>39</sup> et al., found an association between *tinea pedis* and onychomycosis of 20 to 33.8%. In China, Zhang et al.<sup>40</sup>, reported this coexistence in 136 of 236 patients; in Brazil, Avelar et al.<sup>23</sup>, reported a significant association (p<0.001) attributed to sports, use of pools, fomites (socks, towels and shoes) and an increased incidence of diabetes and peripheral vascular disease. Other associations were fingernail onychomycosis and *tinea cruris*, which could be due to self-inoculation, especially when handling infected toenails or result scratching a *tinea corporis* on other localizations.<sup>36,37</sup> In this study, 25% of the patients had suffered trauma, which correlates with other studies<sup>41,42,43</sup>.

The prevalence of onychomycosis is about 2.43% based on previous studies in Guatemala (unpublished data); dermatophytes cause 71% of onychomycosis<sup>13</sup> and the main causal agent is *T. rubrum* with 87% of the cases. *Candida* sp. 20-29% and non-dermatophyte molds 3%<sup>2,4,7</sup>, however, the last ones are considered as secondary invaders<sup>2,44,45</sup>. In the present study, the most frequently observed dermatophyte was *T. rubrum*, which coincides with reports previously made in Guatemala<sup>46</sup>. It was determined that 25% (n = 32/130) of onychomycosis were due to mixed associations (dermatophyte / dermatophyte, dermatophyte / non-dermatophyte mold, dermatophyte / yeast).

In this study, an association between *T. rubrum* and *Candida* sp. was found in 44% of cases, this findings are similar with those reported by Torres-Guerrero et al.<sup>47</sup>, who found



**Graph 1.** Risk factors associated with onychomycosis



**Graph 2.** Fungal agents responsible for mixed onychomycosis

this association in 7 of 11 patients with superficial fungal infections and in 6 of 120 cases of onychomycosis, as well as the finding made by Gelotar et al.<sup>43</sup> who found 1 case of 17, whereas *T. rubrum* was the most common causal agent and *Candida* was the most common opportunistic yeast. *T. rubrum* and *M. canis* were observed in 31.2% of cases; the latter has been reported in Guatemala as an important agent<sup>46,48</sup>, as it has been identified in 10 of 18 cases of *Microsporium* spp. onychomycosis in a period of 42 months<sup>49</sup>; however, it differs with Meric et al.<sup>50</sup> who reported *T. rubrum* as the most common causative agent, followed by *T. mentagrophytes* and *M. canis* which were seen only in 1.9% of cases, therefore, the frequencies of the dermatophyte fungi have an endemic variation. Regarding other associated pathogens, Martínez et al.<sup>51</sup>, reported *Aspergillus*, *Scopulariopsis*, *Cladosporium*, *Fusarium* and *Acremonium* as important causal agents of onychomycosis representing the 78.15% of all opportunistic pathogens<sup>45,51</sup>, which may also be frequently associated, these data are similar to our results in this study, where opportunistic fungi such as *Scopulariopsis* spp., *Aspergillus* spp. and *Fusarium* spp. were associated with dermatophytes in 9.3%, 3.1% and 3.1%, respectively<sup>51</sup>. The importance of the findings in this study is related correlates to choosing an effective treatment or possible prolonged treatment. It should be mentioned that it was not possible to classify the *Candida* species associated with mixed onychomycosis, but it should be noted that it is important to identify them because more than one species can be found associated with mixed onychomycosis. Also, it could not be determined the correlated species of opportunistic fungi since in many cases this entails the use of molecular techniques for their identification and classification.

**Table 4.** Association dermatophytes-opportunistic fungi in mixed onychomycosis

Mixed causative agents	Number	Percentage
<i>T. rubrum</i> + <i>Candida</i> spp.	14	44%
<i>T. rubrum</i> + <i>M. canis</i>	10	31.2%
<i>M. canis</i> + <i>Candida</i> spp.	3	9.3%
<i>T. rubrum</i> + <i>Scopulariopsis</i> spp.	3	9.3%
<i>T. rubrum</i> + <i>Aspergillus</i> spp.	1	3.1%
<i>T. rubrum</i> + <i>Fusarium</i> spp.	1	3.1%
Total	32	100%

## Conclusions

In this study, mixed onychomycosis represented 25% of fungal infections of the nail in Guatemala; this shows the importance of recognizing and treating them properly. Most of the patients were female (68%) between 41 to 60 years old (59.5%); an important relationship between diabetes was observed and the main association was *T. rubrum* with *Candida* spp. The main comorbidities associated with total dystrophy onychomycosis were the cutaneous dermatophytic infections.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this investigation.

**Confidentiality of data.** The authors declare that no patient data appears in this article.

**Right to privacy and informed consent.** The authors declare that no data that enables identification of the patients appears in this article.

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**Conflict of interest:** authors declare no conflict of interest on this paper.

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