

## Epidemiology, Causative Agents and Clinical Features of Onychomycosis in El-Gharbia Governorate

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**O**NYCHOMYCOSIS is a fungal disease of the nails that infects the nail plate, nail bed and matrix, caused by various species of dermatophytes, yeasts and molds. The aim of this investigation is to determine the clinicomycological and epidemiological profile of the etiologic agents of onychomycosis. The study population included 100 suspected cases of onychomycosis referred to the outpatient clinic at the Department of Dermatology at Tanta University Hospital, El-Gharbia Governorate, Egypt during 2015-2016. Nail clippings were collected in sterile Petri dishes for direct microscopic examination and culture. Fungal isolates were identified. The results showed that candidal onychomycosis was the most common clinical type. *Candida tropicalis* was the most prevalent causative species. It was more common among females aged 41–50 years. The incidence was higher during summer and autumn seasons. Housewives were the commonest infected population. Fingernails were affected more frequently than toenails. The pattern of causative agents and clinical signs of onychomycosis is altering region to region, so repeated epidemiological surveys of onychomycosis seems to be fundamental. The present study provided novel and appropriate epidemiologic data of onychomycosis for the better prevention and treatment of this fungal infection.

**Keywords:** Onychomycosis, Dermatophytes, *Candida*, Epidemiology.

### Introduction

Onychomycosis is a common fungal infection affecting both fingernails and toenails which usually caused by dermatophytes, yeasts and molds (Faergemann & Baran, 2003). Onychomycosis occurs worldwide and accounts for upto 50% of all nail infections and 30% of all superficial fungal infections of the skin (Seebacher et al., 2008).

Onychomycosis is classified into five clinical types, according to the fungal invasion of the nail: Distal and/or lateral subungual onychomycosis (DLSO) which appeared when onycholysis, discoloration, subungual hyperkeratosis and thickening affecting the distal and/or lateral nail. White superficial onychomycosis (WSO) is white spots on or in the nail plate with textural changes. Proximal subungual onychomycosis (PSO), appeared if discoloration and onycholysis affected the proximal part of the nail. Candidal onychomycosis (CO) is characterized by markedly thickened and roughened nail plates. Total dystrophic onychomycosis (TDO) involves the entire nail bed and nail plate with thickening and roughening or destruction of nail plate (Rafiq, et al., 2013).

Dermatophytes are the main causative agent in temperate climates and account for 90% of toenail infections and at least 40% of fingernail infections (Singal & Khanna, 2011). *Trichophyton rubrum* is the most common isolated dermatophyte followed by *T. mentagrophytes* and *T. tonsurans* (Seebacher et al., 2007 and Veer et al., 2007). Fingernail onychomycosis is more commonly associated with yeast infection, particularly *Candida* spp. (Meireles et al., 2008). The main nondermatophyte molds involved in onychomycosis are *Scopulariopsis*, *Aspergillus*, *Fusarium*, *Penicillium* and *Scytalidium* species (Narain & Bajaj, 2016).

The prevalence of onychomycosis varies in different geographical areas due to several factors such as climate, socio-economical, occupational situations, aging, life-style and habits (Perea et al., 2000 and Sigurgeisson & Steingrimsen, 2004).

In Egypt, higher priorities directed to other diseases, had resulted in low awareness of onychomycosis by physicians (El-Said, 2002). So, few data are available regarding the prevalence of onychomycosis. Therefore, this study was carried out to shed lights on the various clinical patterns, aetiologic agents and to correlate the clinical patterns with causative pathogens.

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## Materials and Methods

### Study group

One hundred specimens were collected as nail clippings or nail debris from patients clinically diagnosed to have onychomycosis by specialists of dermatology. This was achieved by visiting the outpatient clinic of the Dermatology Department twice a week during a year from March 2015 to February 2016 at Tanta University Hospital, El-Gharbia Governorate, Egypt. All patients provided an informed consent to participate in this study. The study was approved by the Ethics Committee of faculty of Medicine Tanta University.

The assessment of patients was conducted and consisted of an interview, clinical examination and collection of specimens for microbiological studies. All subjects completed a questionnaire that contained a demographic data, patient history, specific data related to risk factors for onychomycosis (age, gender and occupation) the clinical appearance according to site of lesion (DLSO, PSO, SWO, TDO and CO) and location of onychomycosis (toenail, fingernail).

### Specimen collection and processing

The specimens were obtained from clinically affected nails, by a vigorous scrapping of the nail bed, the underside of the nail plate and the hyponychium, after cleaning the affected area with 70% ethanol. Samples from each patient were placed in separate sterile Petri dish. All specimens were analyzed by direct microscopy and culture. Scales scraped from the nails were analyzed for fungal elements, such as hyphae or blastoconidia, by direct microscopy examination, in potassium hydroxide (20%) (Rafiq et al., 2013).

### Culture and identification

All nail samples were inoculated on each

of two isolation media (1) Sabouraud dextrose agar with 5% chloramphenicol (SDA, HiMedia laboratories) (2) SDA with 5% chloramphenicol and cycloheximide in duplicate. Cultures were incubated at 25°C and 37°C and examined daily for six weeks. The identification of dermatophytes and non-dermatophyte moulds was performed by macroscopic and microscopic morphological examination of colonies as described by De Hoog et al. (2000) and Robert & Pihet (2008). Yeasts isolates were identified by growth on Brilliance *Candida* agar (Oxoid Ltd., Hampshire, United Kingdom). Plates without growth, even after 4 weeks of incubation were considered negative. All the samples of dermatophytes were stained with Lactophenol cotton blue and then photographed.

### Statistical analysis

Statistical analysis was carried out using a Pearson chi-square test to evaluate the significance of all the studied factors and the spread of onychomycosis.

## Results

A total of 100 patients with suspected onychomycosis were enrolled during the present study period. Of these patients, 86 (86%) were females and 14 (14%) males as in Fig. 1. The prevalence of onychomycosis in different age groups showed that the patients ranging from 41 to 50 years (40%) were the most commonly affected (Table 1). Table 2 and Fig. 2 showed the various clinical types and sites of nail involvement. The most common clinical type was candidal onychomycosis (52%), followed by distal-lateral subungual onychomycosis (35%), total dystrophic onychomycosis (11%) and the least common was proximal subungual onychomycosis representing (2%).

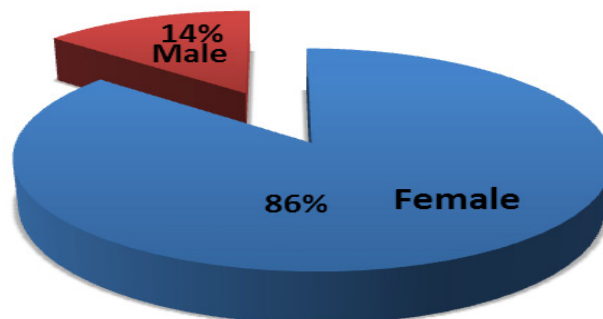


Fig.1. Percentage of onychomycosis among different sexes during the year of the survey (2015).

**TABLE 1. Distribution of onychomycosis among different sex and age groups.**

Age Group	Percentage of cases		Percentage of total cases
	Sex		
	Female	Male	
≤10	2	0	2
11-20	2	0	2
21-30	8	0	8
31-40	13	5	18
41-50	34	6	40
51-60	17	0	17
>60	10	3	13
Percentage of total cases	86	14	100
Chi-square $\chi^2$		8.482	
p-value		0.205	

Statistical significant difference ( $P < 0.05$ )

**TABLE 2. Classification of onychomycosis according to clinical types.**

Clinical type	Nail site		Total cases
	Toenail	Fingernail	
CO	0	52	52
DSO	24	11	35
PSO	1	1	2
TDO	7	4	11
WSO	0	0	0
Total	32	68	100
Chi-square $\chi^2$		51.340	
p-value		0.00*	

\* Statistical highly significant difference ( $P < 0.001$ )



**Fig.2. Different types of onychomycosis. (A) DLSO; (B) CO; (C) TDO; and (D) PSO.**

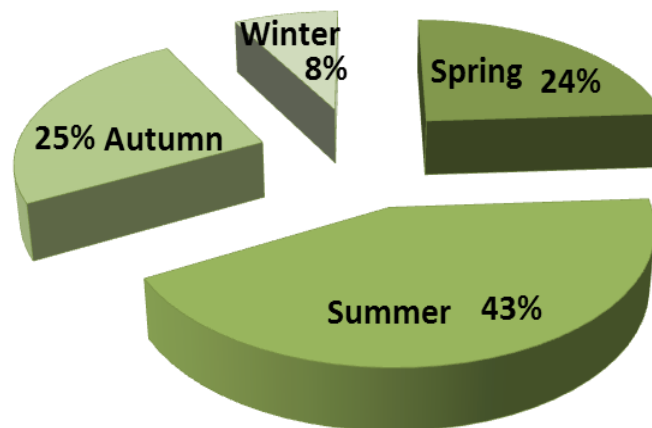
The data represented in Table 3 showed that onychomycosis was significantly more common among housewives (72 cases), followed by manual workers (16 cases) and farmers (7 cases), but less common among students (5 cases). The highest

incidence of onychomycosis was observed in summer (43 cases), followed by autumn (25 cases). Spring (24 cases) and winter (8 cases) showed a low incidence of onychomycosis (Fig. 3).

**TABLE 3. Distribution of onychomycosis among different occupational careers during year of survey (2015).**

Occupational career	No. of cases
Housewives	72
Manual workers	16
Farmers	7
Student	5
Total	100
Chi-square $\chi^2$	120.560
p-value	0.00*

\*Statistical highly significant difference ( $P < 0.001$ )



**Fig. 3. Seasonal incidence of onychomycosis during the year of the survey (2015).**

Fingernail onychomycosis was recognized in 68% of cases and toenail onychomycosis in 32%. The rate of affected fingernails was higher in females in which yeasts were the most isolated agent (Table 4).

Fungal isolates causing onychomycosis in the present study were grouped into yeasts (56%), dermatophytes (36%) and non dermatophytes was detected in 8% (Fig. 4). This means that yeasts were the most common aetiological agent causing onychomycosis.

Table 5 showed that the most common yeast causing onychomycosis was *Candida tropicalis*

(20%) followed by *C. parapsilosis* (10%), *C. glabrata* (8%), *C. albicans* (8%) and *C. krusei* (6%) and *Rhodotorula* species (5%). Also, the most commonly isolated dermatophyte was *Trichophyton mentagrophytes* (17%), followed by *T. tonsurans* (14%) and *T. verrucosum* (5%). Among the nondermatophyte moulds, *Aspergillus niger* (4%), *Aspergillus flavus* and *Chrysosporium* sp. were isolated from 2 patients.

Figure 5 represented the appearance of different *Candida* spp. on Brilliance *Candida* agar. Also Fig. 6 showed the macroscopic and microscopic appearance of dermatophytes and

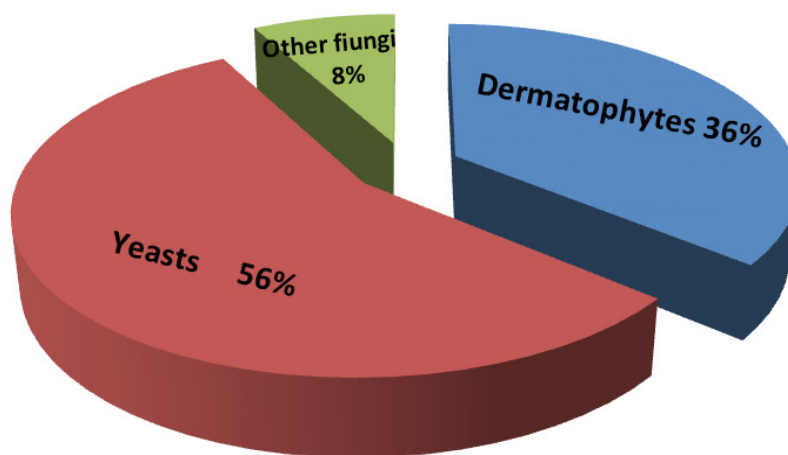
nondermatophytes causing onychomycosis. Culture of *Trichophyton mentagrophytes* (Fig. 6-1A) showed flat colonies with central folding, white to cream in color, and downy surface, with yellow-brown reverse pigment. Microscopic appearance (Fig. 6-1B) showed branched conidiophores bearing numerous grape-like clusters of spherical microconidia. *T. tonsurans* (Fig. 6-2A) showed flat powdery yellow colonies, Fig. 6-2B showed balloon shape of microconidia. *T. verrucosum* (Fig. 6-3A) showed gray-white flat colonies, Fig. 6-3B showed globose microconidia produced on stalks. *A. niger* (Fig. 6-4A) showed

colonies consist of dark-brown to black conidial heads, Fig. 6-4B showed large conidial heads, globose dark brown conidia. *A. flavus* (Fig. 6-5A) showed yellow to green color, flat granular colonies, Fig. 6-5B showed conidial heads are typically radiate, rough conidiophores covering the entire vesicle, and phialides point out in all directions. Conidia are globose to subglobose. *Chrysosporium* sp. (Fig. 6-6A) showed flat powdery orange color colonies which grow on cycloheximide, Fig. 6-6B conidia were typically pyriform to clavate with truncate bases.

**TABLE 4. Distribution of fungal groups causing onychomycosis in various nail sites.**

Type	No. of cases						Total
	Fingernail			Toenail			
	Male	Female	Total	Male	Female	Total	
Dermatophytes	5	7	12	6	18	24	36
Yeasts	0	52	52	2	2	4	56
Filamentous fungi	1	3	4	0	4	4	8
Total	6	62	68	8	24	32	100
Chi-square $\chi^2$		22.423			2.667		13.269
p-value		0.00*			0.264		0.001*

\* Statistical significant difference (P < 0.05)

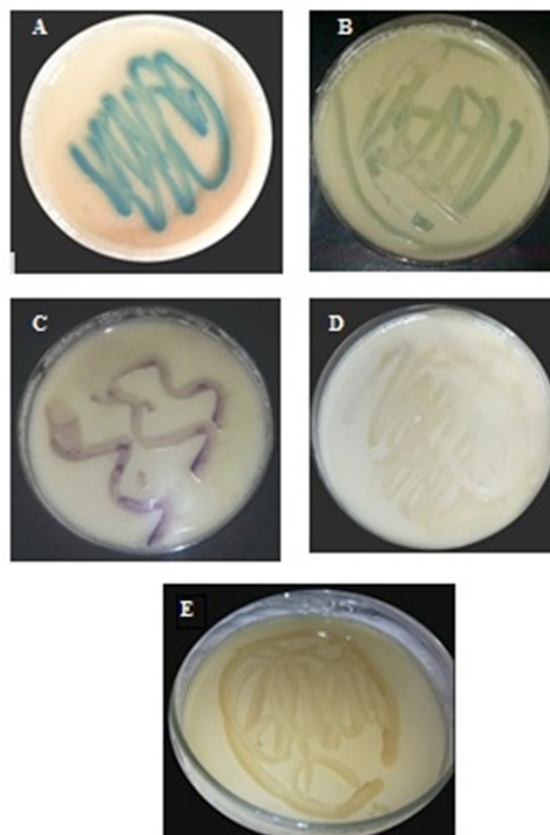


**Fig.4. Causative agents of onychomycosis during the year of the survey (2015).**

**TABLE 5. Fungal isolates causing onychomycosis during the year of study (2015).**

Fungal isolates	Percentage of cases
<b>Yeasts:</b>	
<i>C. tropicalis</i>	20
<i>C. parapsilosis</i>	10
<i>C. glabrata</i>	8
<i>C. albicans</i>	8
<i>C. krusei</i>	6
<i>Rhodotorula</i> sp.	4
<b>Dermatophytes:</b>	
<i>T. mentagrophytes</i>	17
<i>T. tonsurans</i>	14
<i>T. verrucosum</i>	5
<b>Nondermatophytes:</b>	
<i>Aspergillus niger</i>	4
<i>Aspergillus flavus</i>	2
<i>Chrysosporium</i> sp.	2
<b>Total</b>	100
<b>Chi- square <math>\chi^2</math></b>	45.68
<b>p-value</b>	0.00*

\* Statistical highly significant difference ( $P < 0.001$ )



**Fig.5. Phenotypic characteristic of *Candida* spp. on Brilliance Candida agar.**

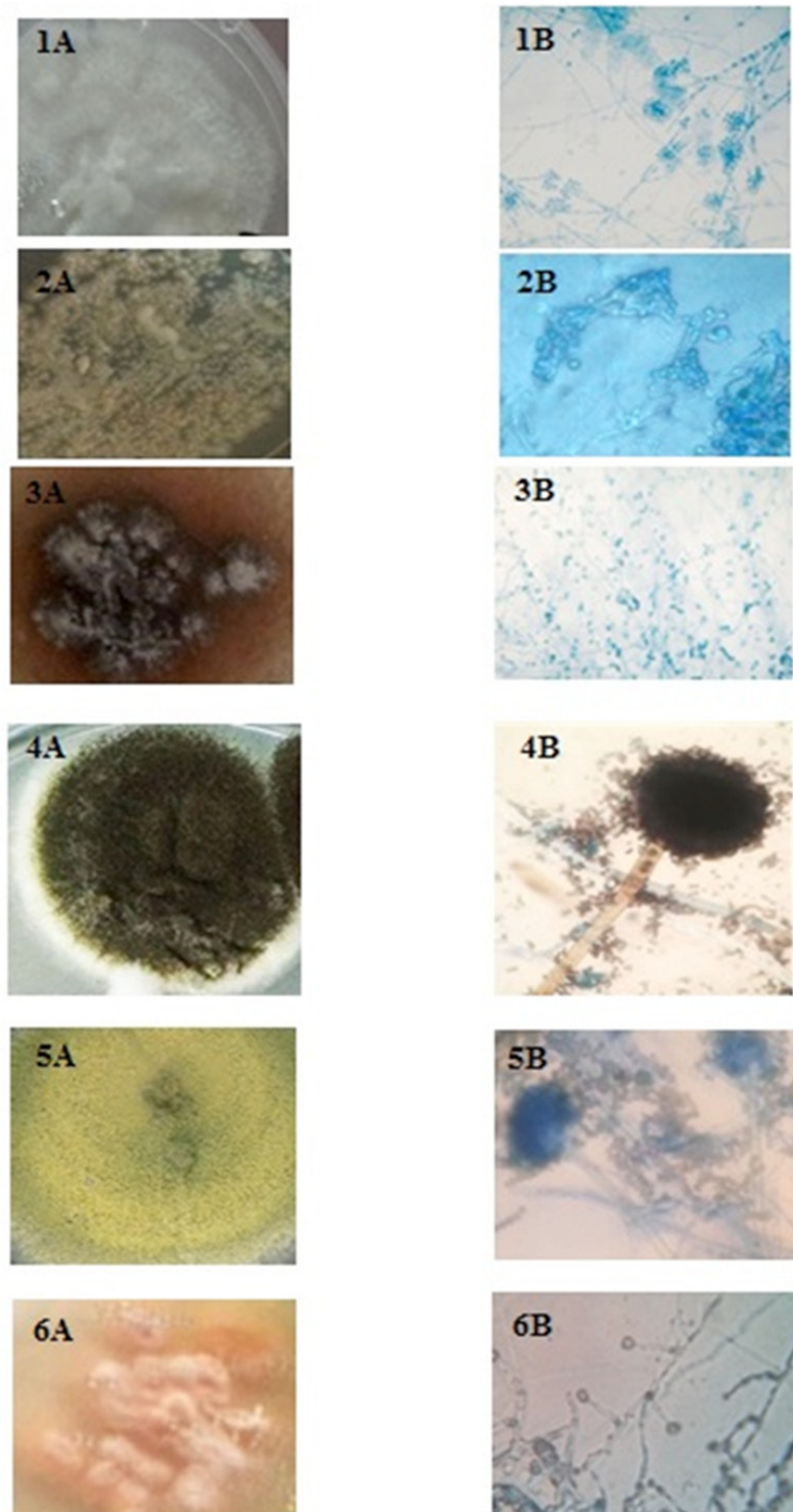


Fig. 6. Macroscopic and microscopic appearance of dermatophytes and nondermatophytes causing onychomycosis. (A) Culture and (B) microscopic appearance. (1) *T. mentagrophytes*; (2) *T. tonsurans*; (3) *T. verrucosum*; (4) *Aspergillus niger*; (5) *Aspergillus flavus* and (6) *Chrysosporium* sp.

## Discussion

Onychomycosis had been observed to be one of the most common nail diseases in patients applying to the outpatient department of dermatology at Tanta University Hospital, Tanta, Egypt. In the present survey, a total of 100 cases were recorded with onychomycosis over 12 months (March 2015–February 2016).

In the present study, onychomycosis was found to be more common in females (86%) than males (14%). A similar female predominance (62.9%) was reported by Afshar et al. (2014). This can be attributed to the fact that females do household wet work as laundry and house cleaning. On the other hand these results differ from some other studies which showed that onychomycosis was more common in males than females (Ahmad et al., 2010). In the present survey, onychomycosis affects all age groups. The most cases found in patients aged between 41 and 50 years. Similar observation was reported by Jayatilake et al. (2009) who observed that onychomycosis prevalence increases with age reaching its maximum 40-60 years old. On the contrary Grover (2003) reported a high prevalence in the age group of 20-40 years.

Housewives formed the largest group (72%) in our study and this could be due to the involvement of housewives in domestic activities that include wet work like cleaning and laundry. They are also more concerned about cosmetic appearance of their nails. Similar results were reported by Chadeganipour et al. (2008) who found that housewives are the commonest. Other study carried out on 182 patients from 2006 to 2007 at Kathmandu, Nepal reported that housewives represent 28.5% of their patients (Neupane et al., 2009). This result was in contrast with Mudita et al. (2007).

In our study, fingernails were involved in 68% of patients while, toenails were involved in 32% of patients. These results were nearly similar to the results of a study in Indonesia (Bramono & Budimulja, 2005), where fingernails were involved in 56.5% of patients and toenails in 17% of patients. On the other hand Mudita et al. (2007) found that toe nails were affected in 56.9%, finger nails in 32.3% and both nails in 10.8% of patients. The low incidence of toenails onychomycosis in our country may be attributed to open footwear and less concern for appearance of feet and toe nails in our people.

The predominant clinical type of onychomycosis in our study was candidal onychomycosis (52%)

and was seen among patients whose occupations involved wet work followed by distal-lateral subungual onychomycosis (35%) and the least common was total dystrophic onychomycosis (11%). Reports from India showed that candidal onychomycosis (58.82%) was the predominant clinical type followed by distal subungual onychomycosis (38.72%) (Jesudanam et al., 2002).

In the present study the most frequently isolated fungal groups were yeasts 56% followed by dermatophytes in (36%) and non-dermatophyte moulds (8%). Higher isolation rate of *Candida* species (52%) noted in this study came in harmony with those of Wajid et al. (2016) and Jesudanam et al. (2002). The most common isolated species in the present survey was *Candida tropicalis* (20%). A similar result had been reported from Turkey (Manzano-Gayosso et al., 2011). On the other hand Abdo & Abd Elrahem (2016) reported that dermatophytes were the most prevalent species (72.73%) followed by non-dermatophytic molds. *Trichophyton mentagrophytes* was the most common dermatophyte isolated (17%). Similar finding was reported from Iran (Aghamirinia & Ghiasian, 2010). In India, Sarma et al. (2008) reported that *T. rubrum* was the most common dermatophyte associated with onychomycosis.

The frequency of fungal infections varies according to seasons. The highest frequency of onychomycosis (43%) was in summer. Our results were similar to those reported by Ismael & Al-Kafri (2016). This can be attributed to the fact that superficial mycoses grow best in warm and humid climate as in tropical and subtropical regions.

## Conclusion

It can be concluded that in El-Gharbia Governorate Egypt, the main cause of onychomycosis are yeasts followed by dermatophytes and a small number of cases are caused by filamentous fungi other than dermatophytes. Due to the long duration of therapy required for onychomycosis treatment regimen, it is important to increase awareness of people to protect themselves from infection. The results of our work don't differ significantly from those observed by previous researchers around the world, and any variation is probably due to the differences in climates and lifestyle conditions of the different study populations.

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## الوبائية، العوامل المسببه والسمات السريره للإلتهاب الفطرى للأظافر فى محافظة الغربية

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الإلتهاب الفطرى للأظافر هو عدوى فطريه تصيب صفيحة الظفر وفرش الظفر ومنبت الظفر وينتج عن أنواع مختلفة من الفطريات الجلدية والخمائر والفطريات الخيطيه الأخرى من غير الفطريات الجلدية. الهدف من هذه الدراسة هو التشخيص الإكلينيكي والمعملى وكذلك عزل وتعريف الفطريات المسببه لهذا المرض شملت الدراسة 100 حالة مشتبه فى إصابتها بالإلتهاب الفطرى للأظافر من المترددين على العيادة الخارجية لقسم الأمراض الجلدية فى مستشفى جامعة طنطا فى محافظة الغربية بمصر فى خلال الفترة من 2015-2016. جمعت قصاصات الأظافر فى أطباق بترى معقمة للفحص الميكروبيولوجى. تم تعريف العزلات وأظهرت النتائج أن الإلتهاب الفطرى للأظافر بالخمائر هو النوع الأكثر شيوعا وكانت كانديدا تروبيكالييس هى النوع الأكثر إنتشارا. وكان المرض أكثر شيوعا بين الإناث التى تتراوح أعمارهم بين 41-50 سنة وكان معدل الإصابة أعلى فى فصلى الصيف والخريف وكانت ربات البيوت هن الأكثر إصابة . وأظافر اليد أكثر إصابة من أظافر القدم. وحيث ان العوامل المسببه لهذا المرض تتغير من منطقة لمنطقة وأذلك الدراسة الإستقصائية لهذا المرض لابد أن تكون مستمرة. وقد وفرت هذه الدراسة بيانات مناسبة للإلتهاب الفطرى للأظافر من أجل الوقاية من هذه العدوى الفطرية بشكل أفضل.