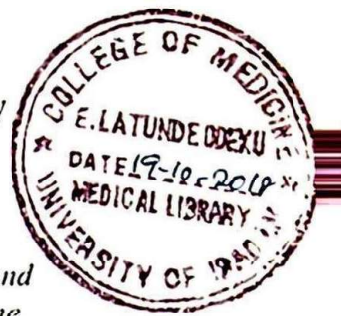


Onychomycosis in patients attending a dermatology outpatient clinic in Lagos, Nigeria

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Abstract

Background: Onychomycosis refers to fungal infection of the nails either as a primary event or secondary infection of a previously diseased or traumatized nail. Some of the risk factors associated with onychomycosis include advancing age; smoking; peripheral arterial disease; diabetes mellitus (DM) and immunosuppression.

Aim: The work aims to determine the clinical characteristics; predisposing factors, causative organisms in patients with onychomycosis who attended the dermatology clinic in Lagos University Teaching Hospital between July 2013 and Jan 2014.

Methods and Materials: This is a prospective observational study. All consenting patients with clinical features suggestive of superficial fungal infections were recruited. Data was collected using a structured questionnaire that contains bio- and socioeconomic data, clinical diagnosis, underlying disease condition and possible predisposing factors. Nail clippings and skin scrapings were taken for KOH microscopy and culture which were by standard methods.

Result: Onychomycosis was found in 19.0% of the patients recruited. The infection was most common between the ages of 30 and 39 (21.9%). Finger nail infections were more common affecting 16 (61.5%) patients than toenails 6 (23.1%); and combined toe and fingernail infections were found in 4 (15.4%). Dermatophytes accounted for 73.1% (19) of the isolates. The yeast *Candida albicans* (17.5%) and the dermatophyte *Trichophyton rubrum* (17.5%) were shown to be the most common pathogens of onychomycosis. Onychomycosis was more common in patients with HIV infection (25.0%).

Conclusion: Onychomycosis was most commonly seen in the adults in this study as opposed to the elderly in other climes and *T. rubrum* and *C. albicans* were the most commonly cultured organisms.

Keywords: Onychomycosis, fungal infections, risk factors, *trichophyton rubrum*, *candida albicans*

Résumé

Contexte : L'onychomycose réfère à une infection fongique des ongles soit comme un événement primaire, ou comme une infection secondaire d'un ongle préalablement malade ou traumatisé. Certains des facteurs de risque associés à l'onychomycose comprennent l'âge avancé; le tabagisme; la maladie artérielle périphérique; le diabète sucré (DM) et l'immunosuppression.

But: Le travail vise à déterminer les caractéristiques cliniques; facteurs prédisposant, organismes causatifs chez les patients atteints d'onychomycose qui ont accédé à la clinique de dermatologie de l'Hôpital d'Enseignement Universitaire de Lagos entre Juillet 2013 et Janvier 2014.

Méthodes et matériaux : Ceci est une étude observationnelle prospective. Tous les patients consentants présentant des signes cliniques suggestifs d'infections fongiques superficielles ont été recrutés. Les données ont été recueillies à l'aide d'un questionnaire structuré qui contient des données bio- et socio-économiques, un diagnostic clinique, l'état pathologique sous étendu et d'éventuels facteurs prédisposant. Des coupures d'ongles et des raclures cutanées ont été prises pour la microscopie et la culture au KOH qui étaient par des méthodes standard.

Résultat: L'onychomycose a été retrouvée chez 19,0% des patients recrutés. L'infection était plus fréquente entre 30 et 39 ans (21,9%). Les infections des ongles des doigts étaient plus fréquentes touchant 16 (61,5%) patients que les ongles des orteils 6 (23,1%); et une infection combinée de l'orteil et de l'ongle ont été trouvées chez 4 (15,4%). Les dermatophytes représentaient 73,1% (19) des isolats. La levure

Candida albicans (17,5%) et le dermatophyte *Trichophyton rubrum* (17,5%) se sont avérés être les pathogènes les plus communs de l'onychomycose. L'onychomycose était plus fréquente chez les patients infectés par le VIH (25,0%).

Conclusion: L'onychomycose était le plus souvent observée chez les adultes de cette étude, par opposition aux personnes âgées dans d'autres régions et *T. rubrum* et *C. albicans* étaient les organismes les plus couramment cultivés.

Mots - clés : Onychomycose, infections fongiques, facteurs de risque, *trichophyton rubrum*, *candida albicans*

Introduction

Onychomycosis refers to fungal infection of the nails either as a primary event or secondary infection of a previously diseased or traumatized nail. Infection could be caused by a dermatophyte, yeast or non-dermatophyte mould species and the clinical appearance may indicate the nature of the causative organism. The term 'Tinea unguium' which means dermatophyte infections of the nail has been used synonymously but erroneously with onychomycosis. Tinea unguium represents 90% of onychomycosis of the toe nails and 50% of that of the finger nails [2]. Onychomycosis, though not life threatening, causes symptoms such as pain, discomfort and disfigurement which impair activities of daily living and negatively impact the quality of life of affected individuals. [3-5] Onychomycosis constitutes 50% of all nail problems and about 30% of all dermatophyte infections [1].

Onychomycosis affects all races but there is wide geographical and ethnic variation in the causative species. Approximately 20% of individuals aged between 40 and 60 have onychomycosis [6,7]. A recent review on the global burden of onychomycosis revealed that the mean prevalence in Europe and North America was 4.3% in the population-based studies, but was 8.9% for the hospital-based studies [8]. Both population-based and hospital-based studies showed that onychomycosis is more common in toenails and is seen more frequently in males [8]. Studies from Finland and the UK also showed higher frequency in males than females [1,9]. Two previous hospital based studies done in Lagos, Nigeria revealed the frequency of onychomycosis to be 10.5 and 13.5%; higher than findings in Europe suggesting likelihood of higher prevalence in Africa than in Europe [1,10,11].

Some of the risk factors associated with onychomycosis include advancing age; smoking; peripheral arterial disease; diabetes mellitus (DM); immunosuppression from human immunodeficiency virus (HIV) infection, immunosuppressive therapy, chemotherapy and antibiotic use [12-16]. HIV is associated with extensive onychomycosis which is a marker of declining immunity in HIV infection. [17] Onychomycosis has also been documented as an occupational hazard in farmers who till the soil manually and those who walk bare-footed in developing countries [18]. A genetic basis for onychomycosis has been suspected in a study in which a familial pattern was found in patients with distal subungual onychomycosis [19]. Children of

patients with onychomycosis were noted to harbor the pathogen (*T. rubrum*) on the soles of feet; and this spreads at a later date to the toe nails and other sites on the body [19]. The increased use of health clubs, commercial swimming pools and occlusive footwear have also been implicated in the increasing prevalence of onychomycosis [16].

Dermatophytes are the most commonly implicated group of fungi in onychomycosis, found all over the world. *Trichophyton rubrum* (*T. rubrum*) is the most common aetiological agent of nail infections worldwide and accounts for 70% of onychomycosis, followed by *T. mentagrophytes* (20% of onychomycosis) [1,20]. In a multi-centred study carried out in Australia and New Zealand, non-dermatophyte fungi (yeasts and moulds) were found in 64% of the patients either as a co-infection, secondary infection or contaminants. [21] The most common cause of yeast infection is *Candida albicans* [22].

This work aimed to determine the clinical characteristics; predisposing factors, and identify the prevalent causative organism in patients with onychomycosis who attended the dermatology clinic in Lagos University Teaching Hospital.

Materials and methods

This is a prospective observational study of adult patients who attended the dermatology clinic of the Lagos University Teaching Hospital between July 2013 and Jan 2014. All consenting patients were recruited.

Ethical consideration

Ethical approval was obtained from the research and ethics committee of the Lagos University Teaching Hospital. Each study participant or their next-of-kin gave written informed consent to participate in the study after the study procedure including possible benefits and risks had been explained to them.

All patients with suspected fungal infections who gave informed consent over the study period were recruited into the study. Patients unwilling to participate; children whose parents or guardian did not consent; and those on antifungal therapy and clinically responding to therapy were excluded. Onychomycosis was defined as fungal infection of nail. Tinea unguium was defined as dermatophyte infection of nail; tinea capitis as dermatophyte infection of scalp; tinea corporis as dermatophyte infection of skin; tinea manuum as dermatophyte infection of hands; and tinea pedis as dermatophyte infection of feet.

Data collection

A structured questionnaire was administered to each study participants by the investigators. Information requested included bio- and socioeconomic data, clinical diagnosis, underlying disease condition, hobbies such as swimming, gardening and playing with pets; previous history of dermatophyte infection (duration and drug therapy) and history of close contact with persons/animals that have dermatophyte infections.

Specimen collection

Scrapings or clippings of infected nails were collected after cleaning with 70% ethanol. All nails were scraped with a blunt scalpel from the proximal to the distal end of the nail. The first 4-5 scrapings were discarded (to reduce contaminants and get to viable tissue). All samples were collected in a folded square of paper and transported to the microbiology laboratory for analysis.

Microscopy

The specimen was divided into two parts. Potassium hydroxide solution (40% w/v) was added to a part of the specimen for 2 hours to dissolve the nail keratin and release fungal elements for observation by direct microscopy. Fungal elements were identified as hyphae or yeast cells

Culture

The second part of the specimen collected was inoculated onto Sabouraud's dextrose agar with chloramphenicol and gentamicin; plates were sealed with paraffin wax tape and incubated as duplicate cultures at 26°C and 35°C respectively. Yeasts were identified within 48 hours, and germ tube test was used for presumptive diagnosis of *C. albicans*. Cultures were maintained for 4 weeks for molds. However, they were reviewed twice weekly for growth and colonial morphology, pigmentation and consistency.

Germ tube test

Principle: Strains of *C. albicans* produce germ tubes from their yeast cells when placed in a liquid nutrient environment and incubated at 35°C for 2-3 hours similar to the in vitro state.

Procedure: Using a sterile wire loop, a yeast colony from the Sabouraud agar was inoculated into 0.5mls of human serum in a test tube mixed properly and incubated at 35 – 37°C for 2 – 3 hours. After which a drop of the mixture was transferred to a glass slide, covered with a cover slip and then examined under a light microscope using 10x and 40x

objectives. Sprouting yeast cell that were tube-like outgrowth from the cells (germ tube) were reported as *C. albicans* and non-sprouting yeast cell as non-*C. albicans*.

Coccidioides immitis was identified macroscopically and microscopically. Slides were prepared by tease mount stained with lactophenol blue, sealed with coverslips and examined microscopically. Hyphae seen were thin, hyaline and septate. Thicker side branches gave rise to unicellular, barrel-shaped arthroconidia that alternated with thin-walled, empty disjunct cells. The culture demonstrated glistening off-white glabrous colonies.

All slides were examined and identified (slide preparation) in a class 2 biological safety cabinet.

Data analysis

Quantitative data generated from the questionnaire amongst all patients attending the dermatology clinic of Lagos University Teaching Hospital were entered into an excel spreadsheet. Data were analysed at univariate level using SPSS software. The analysis was done with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). Continuous variables were presented as the mean \pm standard deviation. Categorical variables were presented as actual numbers and percentages or as bar charts. The univariate analysis involved the use of descriptive statistics and graph such as frequency distributions and bar chart.

Results

One hundred and thirty seven patients with superficial fungal infections attending the dermatology clinic of the Lagos University Teaching Hospital (LUTH) between July 2013 and February 2014 were recruited. Forty (29.2%) had abnormal looking finger and toe nails with 1:1 ratio of males/females. There were more abnormal finger nails 26 (65%) compared to toenails 10 (25%) with a ratio of 2.6:1. Four patients (10%) had both abnormal looking finger and toe nails. Onychomycosis accounted for 19.0% of the patients with superficial fungal infections at the dermatology outpatient clinic of LUTH.

Demographic characteristics and underlying diseases of patients recruited

One hundred and thirty seven participants were recruited into the study. Table 1 shows the demographic characteristics of these patients. More than half of the patients 103 (75.2%) were less than 50 years old, while only 35 (25.5%) were 50 years

Table 1-Demographic characteristics of patients studied and underlying diseases

Demographic characteristics	Frequency (n)	Percentages (%)
<i>Age in years</i>		
0 – 9	13	9.5
10 - 19	17	12.4
20 – 29	18	13.2
30 - 39	30	21.9
40 - 49	24	17.5
Above 50	35	25.5
<i>Sex</i>		
Male	67	48.9
Female	70	51.1
<i>Marital status</i>		
Single	37	27.2
Married	100	72.8
<i>Tribe</i>		
Yoruba	81	59.1
Igbo	33	24.1
Hausa	10	7.3
Others	13	9.5
<i>Co existing medical diseases</i>		
Diabetes mellitus	24	17.5
Hypertension	35	25.5
HIV/AIDS	26	18.9
Others	14	10.2
None	38	27.7

and above. The mean age was 36 ± 19.2 years (2SD). There were more females than males, with a ratio of approximately 1.04:1. The underlying diseases in the patients in this study include diabetes mellitus 24 (17.5%); hypertension 35 (25.6%) and HIV/AIDS 26 (18.9%). Others were side effects of use of over the counter topical and systemic steroid abuse.

Clinical assessment/diagnosis of patient were based on location of the lesion, with the highest frequency being dermatophyte infection of the nail (Tinea unguium) 35 (25.5%) followed by that of the glabrous skin (T. corporis) 34 (24.8%) and the least frequently occurring being simultaneous infections of various parts of the body accounting for 10 (7.0%) of studied population (Figure 1).

Laboratory diagnosis of all patients' specimen

Table 2 demonstrates the pattern of aetiological agents of keratinized tissue infections in the studied population. Of the 137 patients studied, 49 (35.8%) had no growth after 4 weeks incubation; *C. albicans* was isolated in 24(17.5%); *T. rubrum* in 29 (21.2%); *T. mentagrophyte* in 13(9.5%) and more than one isolate in 3 (2.2%).

Demographic characteristics of patients with onychomycosis

Nineteen (47.5%) of the participants with nail infections were in the 26 to 50 years age range; 14 (35.0%) were 50 years old and above, and only 1

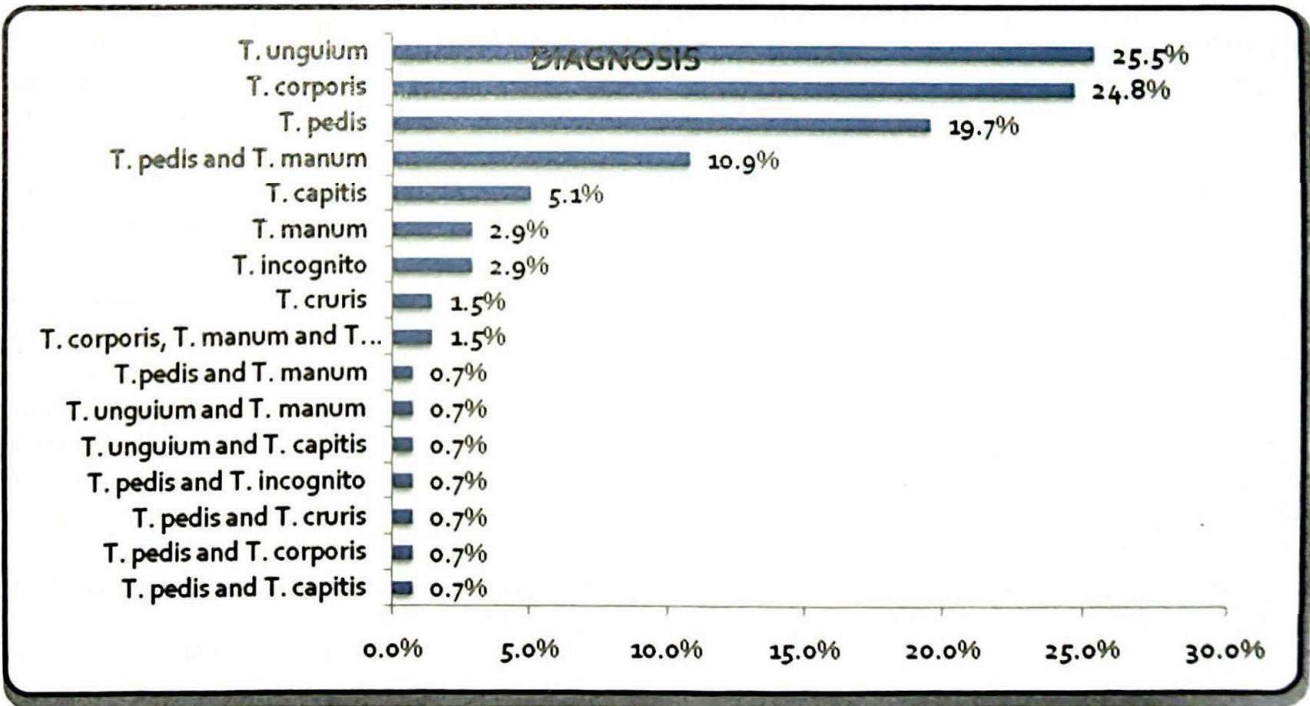


Fig.1: Distribution of clinical diagnosis of studied population

Table 2: Distribution of pathogens causing mycosis

Culture result Isolates	Frequency	Percentage
<i>Coccidioides immitis</i>	1	0.7
<i>Microsporium manum</i> and <i>C. albicans</i>	1	0.7
<i>Microsporium spp</i>	1	0.7
<i>Penicillium notatum</i>	1	0.7
<i>T. rubrum</i> and <i>T. soudanese</i>	1	0.7
<i>T. verrucosa</i>	1	0.7
<i>T. mentagrophyte</i> and <i>C. albicans</i>	2	1.5
<i>T. rubrum</i> and <i>C. albicans</i>	2	1.5
<i>T. scholienii</i>	2	1.5
<i>Microsporium</i> and <i>C. albicans</i>	3	2.2
<i>T. verrucosa</i>	3	2.2
<i>T. soudanese</i>	4	2.9
<i>T. mentagrophytes</i>	13	9.5
<i>C. albicans</i>	24	17.5
<i>T. rubrum</i>	29	21.2
No growth	49	35.8
Total	137	100.0

(7.5%) were between 1 and 10 years of age (Table 1). Mean age was 31 years and male to female ratio was 1:1. There were 26 (19%) cases of onychomycosis. Sixteen (61.5%) were fingernail infections and 6 (23.1%) were toenail infections; combined toe and fingernail infections were 4 (15.3%). Six (15.0%) patients had DM, 4 (10.0%) had hypertension and 10 (25.0%) were HIV/AIDS patients. The patients with DM accounted for five cases of the toenail infections and one case of both toe and fingernail infection. The remaining cases of both finger and toe nail infections were in HIV positive patients.

Eighty five percent presented with only nail infections while the remaining fifteen percent had mixed infections with *T. pedis*, *T. manum* and *T. coporis*.

Isolated causative pathogens of onychomycosis

Direct microscopy with 40% potassium hydroxide (KOH) demonstrated the presence of fungi element in 87% of the samples. Culture isolates demonstrated the most frequent isolates were *T. rubrum* 7 (17.5%) and *C. albicans* 7 (17.5%); 4 samples had more than one organism isolated. Non-dermatophyte moulds were not isolated in this study, (Table 3).

Candida albicans accounted for 10 (56.3%) of all fingernail onychomycosis in this study. The distribution of *C. albicans* was 7 (70%) as pure isolates and 3 (30%) isolated in the mixed growths. Dermatophytes accounted for 7 (43.7%) of cases of

fingernail onychomycosis, with distribution of *T. rubrum* being identified in 4 (57.0) isolates and *T. mentagrophyte* in 3 (43.9%). Isolates from onychomycosis of the toenails were all dermatophytes, with *T. rubrum* accounting for 50% of all isolates.

Table 3: Distribution of aetiological agents of onychomycosis

Culture results Isolates	Frequency	Percentage
<i>Microsporium</i> and <i>C. albicans</i>	1	2.5
<i>T. rubrum</i> and <i>T. soudanese</i>	1	2.5
<i>T. scholienii</i>	1	2.5
<i>T. soudanese</i>	1	2.5
<i>T. verrucosa</i>	2	5.0
<i>T. rubrum</i> and <i>C. albicans</i>	2	5.0
<i>T. mentagrophyte</i>	4	10.0
<i>C. albicans</i>	7	17.5
<i>T. rubrum</i>	7	17.5
No growth	14	35.0
Total	40	100.0

Discussion

Onychomycosis accounted for 19.0% of the cases of dermatophyte infections seen in this study. This is higher than 10.5% and 13.6% obtained from previous reports from this same center; these were however retrospective studies [10,11]. This value is also higher compared to a study from neighboring Cameroun, which gave a prevalence of 8.8%, but lower than that from an Iranian study with a prevalence of 45.2% [23,24]. The variations among these studies may represent real differences in the geographic groups studied, climate, sampling variations, location, clinical type of the infection and other factors [25]. Onychomycosis was more common in those aged 26–50 years in this study. In contrast, Velez *et al.* 1997, and Mercantini *et al.* 1996, reported higher prevalence among adults who were over 50 years of age which was the second most frequent group in these studies [25,26]. This age-related increase of onychomycosis may have resulted from higher probability of nail microtrauma, exposure to pathogenic fungi, and venous insufficiency in older patients as described previously [12,27]. There was only one case of onychomycosis documented in the 1–10 years group in this study, and this is consistent with existing data that demonstrated very low prevalence in children [22,28].

The most common underlying disease in this study was HIV/AIDS. This is consistent with previous reports that demonstrated onychomycosis as a marker of declining immunity in HIV infection and occurs in the early stages of disease progression at CD4⁺ count of > 400 cells/mm³. In the late stages of HIV infection with CD4⁺ count of <200 cells/mm³, patients present with extensive disease: marked subungual hyperkeratosis, severe onychodystrophy, extensive involvement of all nails and profound physical discomfort from periungual inflammation [17]. We were unable to access the CD4 count of participants with HIV/AIDS. These group of patients also accounted for 75% of coexisting finger and toenail infections, which is in keeping with existing data.[17] Diabetes mellitus was also a common underlying condition amongst study participants and this is not surprising considering the fact that it has been estimated that at least one third of DM patients have onychomycosis.[15]

There was no difference amongst sexes in this study, the ratio was 1; 1. However more fingernail lesions were seen compared to toenails, this is not surprising since most times nail infections are viewed as more of a cosmetic problem and fingernails are more visible. Apart from the cosmetic nature of the disease, females in this environment may also present with more fingernail given the cultural background wherein women do more domestic work by hand, enabling chronic exposure of fingers to water.[29]

On direct microscopy, fungal elements were seen in 87% of the confirmed samples, which is relatively higher than documented in other studies; the use of 40% KOH (rather than 20% used in most studies) probably played a role in this. Larger studies will be needed to substantiate this. The results are however in accordance with the findings of a study which was carried by Mikacili and Karimi, which showed positive results of 87.2% by direct microscopy. [24]

The most common organisms which were isolated in culture were dermatophytes (73.1%) and yeasts (17.5%). This finding was in accordance with those of many studies, which had demonstrated a greater prevalence of dermatophytes as the aetiological agents of onychomycosis but it was in contrast to some studies which found yeasts as the most common agents. [1,8,20,14,30] Fingernails accounted for 100% of all yeast infections in this study and most of these patients had concurrent fungal skin infections. A Libyan study also isolated *Candida spp* from 96% of fingernails in women with onychomycosis. [31] *Trichophyton rubrum* was the

most common dermatophyte seen in our study, followed by *T. mentagrophytes*. Although some studies had reported *T. mentagrophytes* as the most common dermatophyte, our finding was in concordance with other studies which found *T. rubrum* as the most common dermatophyte responsible for onychomycosis.[1,8,20] The increased prevalence of *T. rubrum* could have been due to increased virulence and better adaptation to hard keratin of nails.

In conclusion, the most common fungal isolates from onychomycosis in this study were dermatophytes followed by *Candida albicans*. Clinicians, therefore, should inform the general population about onychomycosis prevention. The present findings suggest that clinic, community, and school based onychomycosis prevention programs may benefit the people by addressing the risk factors for onychomycosis infection.

The limitations of this study included the incessant industrial strike action by health care workers that impacted negatively on the sample size. The study was hospital based which might not be a true representation of the burden of the disease in the populace and finally some of the patients may have used herbal/local agents to treat their nails prior to hospital presentation.

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