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Onychomycosis in a group of patients presented to a tertiary care hospital in Sri Lanka.

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Abstract

Background: Onychomycosis is increasingly found in tropical populations.

Objectives: To investigate the incidence and etiology of onychomycosis in a group of patients presented to the Teaching Hospital, Kurunegala, Sri Lanka.

Methods: A total of 47 patients (8 males, 39 females clinically diagnosed with onychomycosis were randomly selected as study participants. After obtaining written informed consent from the participants, demographics and onychomycosis-associated factors were recorded using an interviewer-administered questionnaire. Nail specimens were subjected to direct microscopy (DM) after 20% KOH digestion and were cultured on Sabourauds Dextrose Agar (SDA) containing chloramphenicol alone and SDA with chloramphenicol and cycloheximide. Fungi were identified macroscopically and microscopically.

Results: Out of 47 patients clinically diagnosed with onychomycosis, only 30 (63.8%) were confirmed as onychomycosis mycologically. Overall, 93.4% of confirmed onychomycosis patients (28/30) aged between 20-69 years. Onychomycosis was common among housewives 36.7% (11/30), health care workers 23.3% (7/30) and farmers 16.7% (5/30). Further, 53.3% (16/30) had onychomycosis only in toe nails, 30% (9/30) had only in finger nails. Both toe and finger nails were affected in 16.7% (5/30). Commonest etiology of onychomycosis was dermatophytes 12 (40%) followed by nondermatophytic moulds (NDM) 10 (33%) and *Candida* 8 (27%). Leading pathogenic dermatophytes were *T. mentagrophytes* (41.7%; 5/12) and *T. rubrum* (25%; 3/12). Commonest NDM was *Fusarium* species 60% (6/10).

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Conclusions: Onychomycosis was common in adults (20-69 years) particularly among housewives, health care workers and farmers in our sample. Toenails were the most affected. Etiology of onychomycosis was mainly dermatophytes followed by NDMs and *Candida*. Common dermatophytes associated with onychomycosis were *T. mentagrophytes* and *T. rubrum*. *Fusarium* species was the leading NDM associated with onychomycosis.

Keywords: *onychomycosis, dermatophytes, non-dermatophytic moulds, Candida*

INTRODUCTION

Onychomycosis is one of the leading clinical presentations of superficial mycoses that remain one of the most prevalent human diseases worldwide [1]. For example, it has been reported that approximately 10% of the general population, 20% of the population aged above 60 years, 50% of people aged above 70 years and one-third of diabetic individuals have onychomycosis [2].

Mainly, dermatophytes, non dermatophytic moulds (NDM) and *Candida* are implicated as pathogens in onychomycosis [3,4]. Frequent exposure to above pathogenic fungi, irrational use of antibiotics, HIV infection and immunosuppressive drug therapy, diabetes mellitus, peripheral vascular disease and trauma to the nails are some predisposing factors for this debilitating condition [2,4,5]. Agricultural workers, laborers, housewives, fish mongers, tea makers, athletes and launderers often get onychomycosis depicting an occupational risk [6,7]. High proportion of rural Sri Lankan population is engaged in aforementioned occupations associated with onychomycosis. Meanwhile, comorbidities such as diabetes mellitus has been increasing among Sri Lankans [8]. However, there have been limited studies on the incidence and etiology of onychomycosis in Sri Lankan patients. Therefore, the objective of this study was to investigate the incidence and the etiology of onychomycosis in a group of patients who sought treatment from the Teaching Hospital, Kurunegala, Sri Lanka.

MATERIALS AND METHODS

Study population

A total of 47 patients who were clinically diagnosed as having clinical onychomycosis by a Consultant Dermatologist at the Teaching hospital, Kurunegala, Sri Lanka during the period of December 2014 to March 2015 were randomly selected for the study after obtaining their written informed consent, those who had been treated with antifungals (oral or topical) within last three months were excluded. Ethical clearance was obtained from the ethics review committee of the Medical Research Institute of Sri Lanka (53/2014).

Data collection

Demographic data, medical history, the signs and symptoms regarding onychomycosis were collected using an interviewer administered questionnaire.

Sample collection

Prior to sample collection, affected nails and the fingers were cleaned using 70% alcohol. Full thickness particles of the affected nails were collected for mycological investigations under sterile conditions. Scrapings of the sub-ungual debris and discoloured, brittle or dystrophic nail parts were harvested aseptically and were packed and labeled in a sterile black colour paper and transported immediately for mycological analysis.

Fungal identification

Samples were subjected to mycological investigations and the fungal identification was made based on the macroscopic and microscopic features as described previously [9,10].

Direct microscopy

All the specimens were subjected to direct microscopy after 10-20% KOH digestion. Accordingly, nail scrapings were immersed in a few drops of 10-20% KOH on a glass slide. Nail particles were immersed in 20% KOH solution for 2 hours. Once the specimens softened adequately they were covered with glass cover slips and the excess

KOH was removed. Finally, the specimens were examined under ×10 and ×40 magnifications using the light microscope.

Fungal culture

All the samples were cultured on Sabourauds dextrose agar (SDA) containing chloramphenicol alone and SDA containing both chloramphenicol and cyclohexamide and incubated at 26 ℃. Culture plates were examined every other day for fungal growth up to 3 weeks before they were declared as negative. The fungal growth was noted for colony characteristics in terms of growth rate, texture, surface colour and colour on the reverse side of the culture plate and diffusible pigments. Positive cultures were further examined with lactophenol cotton blue mounts and slide cultures were performed when required. Identification of the isolate was confirmed microscopically with the characteristic features such as macroconidia, microconidia, and the spiral hyphae [12].

RESULTS

Demographic data

Out of 47 patients with clinical onychomycosis 30 (24%) patients (mean age 51.4 years; 05 males, 25 females) were mycologically confirmed as having onychomycosis. When the occurrence of onychomycosis according to the age was considered, 93.4% (28/30) of the patients belonged to 20-69 years group with high prevalence in 20-29 year and 50-59 year age groups (Figure 1A). When the occupations of the participants were considered, 36.7% (11/30) were housewives, 23.3% (7/30) were health care workers and 16.7% (5/30) were farmers (Figure 1B). Further, 53.3% (16/30) had onychomycosis in toe nails, 30% (9/30) had in finger nails. In 16.7% (5/30) patients, both toe and finger nails were affected.

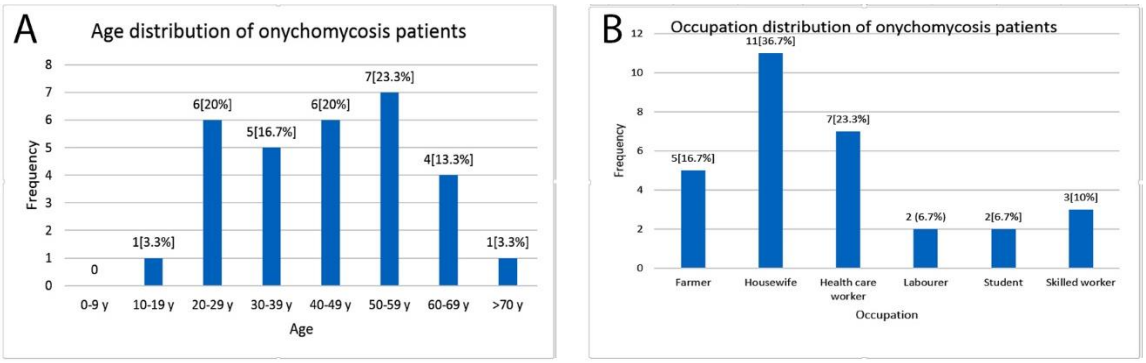

Figure 1. Age (A) and occupation (B) distribution of the onychomycosis patients.

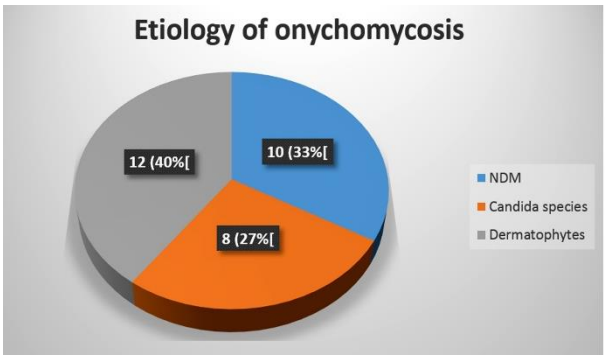

Figure 2. Etiology of onychomycosis

Detailed investigation of the mycologically confirmed onychomycosis samples revealed three main etiological agents. The commonest pathogen was dermatophytes 40% (12/30) followed by NDMs 33% (10/30) and *Candida* 27% (8/30) (Figure 2).

Onychomycosis due to dermatophytes (Tinea unguim)

From the samples collected from 12 patients who had onychomycosis due to dermatophytes (Tinea unguim), 6 samples were both direct microscopy and culture positive. There were 4 samples that produced only positive cultures. Remaining 2 samples showed only typical direct microscopic appearance of dermatophyte in the KOH smear (refractile smooth undulating branching and septate hyaline hyphal filaments of uniform width having arthroconidiospores) (Figure 3A). Different dermatophyte species were identified based on the microscopic features of the samples (Table 1 and Figure 3A, 3B, 3C).

Onychomycosis due to NDMs and *Candida*.

Among onychomycosis due to NDMs, 60% (6/10) was caused by *Fusarium* species (Figure 4). Moreover, *A. niger* (20%) and *A. flavus* (20%) were isolated in two samples each. There were 8 patients with *Candida* onychomycosis.

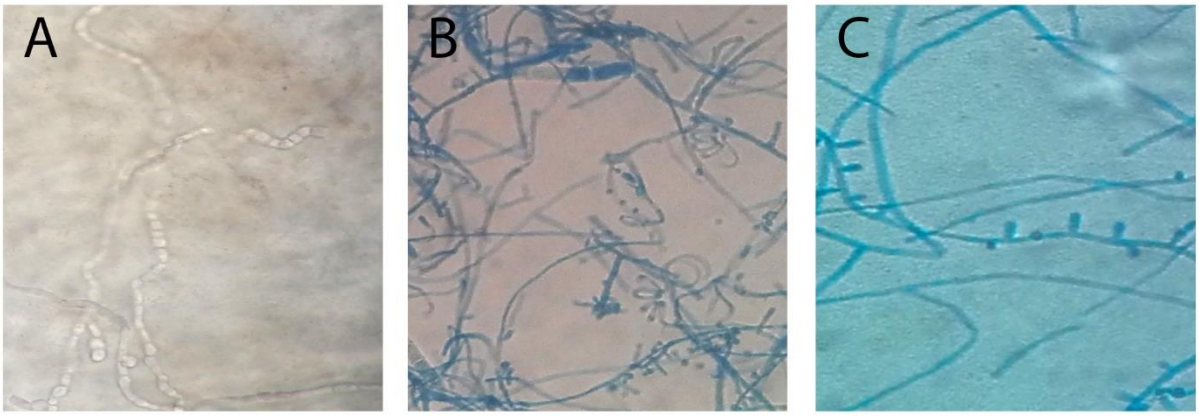

Figure 3. Microscopic features of dermatophytes found in onychomycosis patients; A- characteristic appearance of refractile smooth undulating branching and septate hyaline hyphal filaments of uniform width having arthroconidiospores, B-lactophenol cotton blue smear appearance of *T. mentagrophytes* showing characteristic spiral hyphae, C- lactophenol cotton blue smear appearance of *T. rubrum* showing characteristic tear drop microconidia

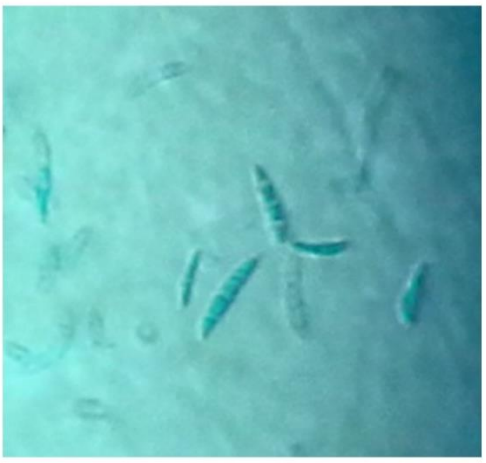

Figure 4. Macroconidia of *Fusarium species*.

DISCUSSION

Onychomycosis represents a considerable proportion of superficial mycoses and it often becomes a nidus for recurrent infections [2]. In the current cohort of 47 patients diagnosed clinically as onychomycosis, only 63.8% (30/47) were confirmed mycologically. As such, there were 36.2% (17/47) who did not produce mycological evidence for onychomycosis despite the clinical diagnosis. Disparity between the clinical and mycological diagnosis of onychomycosis has been noted previously as well. For instance, no mycological confirmation was made with regard to 33.6% and 51.1% of patients, clinically diagnosed as onychomycosis respectively in local as well as overseas studies [11, 12]. Whereas negative fungal growth has been attributed to some unculturable fungi, onychomycosis mimics several other nail pathologies like nail dystrophy, chronic paronychia, viral warts, chronic dermatitis, lichen planus, or psoriasis [13]. On the other hand, sensitivity and the specificity of the mycological tests may also play a role in accurate diagnosis. Current observations agree with El Sayed et al. [14] who have pointed out that accurate diagnosis of onychomycosis exclusively based on clinical grounds is difficult. Accurate diagnosis of the causal agent of onychomycosis should be supported by both direct microscopy and mycological confirmation [4]. In this context, repeated sampling in a bigger patient population may help clarify the rate of onychomycosis confirmed both clinically and mycologically in different cohorts.

Several studies have shown male preponderance to onychomycosis [7, 15,16]. However, women were predominantly affected (39/47) by onychomycosis in the current population. Our observation of female preponderance is in par with some previous studies also [11, 17]. The possible explanation of the female preponderance in our study could be due to the fact that health seeking behavior of men is poor compared to their counterparts [18]. Also, more females being housewives were engaged in household activities that expose them to moisture, soil and trauma predisposing them to nail infections. Further studies using wider sample size is warranted to explore the exact gender predisposition with regard to onychomycosis in Sri Lankan settings.

Onychomycosis was common in adults (20-69 years of age) and it was comparable with the previous studies [19]. Frequent exposure to nail trauma, outdoor activities, large nail surfaces and the presence of comorbidities like diabetes, peripheral vascular disease could be the possible reasons for the adults to have onychomycosis. Onychomycosis was rare in the age group less than 20 years resembling the findings of other studies [11, 17]. Increased nail growth, low surface area of the nail and the presence of less comorbidities like peripheral vascular disease and diabetes may have resulted low incidence of onychomycosis in the younger population. It is important to assess the presence of above comorbidities in the onychomycosis patients with a view to confirm the exact predisposing factors in future studies.

Considering the occupations of the patients affected with onychomycosis, majority were housewives (36.7%) followed by health care workers (23.3%) and farmers (16.7%). Generally, housewives handle water extensively and their nails are prone to trauma during day-to-day household activities such as cooking, cleaning and gardening. On the other hand, females tend to seek treatment for nail pathologies owing to esthetics and their increased health seeking behavior [18]. Onychomycosis in health care workers may be due to wearing shoes, boots and socks for prolonged periods of time and sharing of surgical boots and slippers, especially in clinical settings such as intensive care units and operating theaters. Farmers are exposed to soil, fertilizer, agrochemicals, animals, water and frequent nail trauma during their routine work. However, further detailed studies are necessary to confirm the association between occupations and the incidence of onychomycosis.

When the nails affected by onychomycosis in the current sample were considered, it was noted that 53.3% patients had toe nails affected while 30% had finger nails affected. Both toe and finger nails were affected in 16.7% patients. Higher incidence of onychomycosis in the toe nails has been reported previously too [2, 15]. Some studies have indicated that fingernails were commonly affected by onychomycosis [12]. It is likely that vulnerability for repeated trauma, wearing socks shoes and boots, high exposure to soil and moisture

compared to finger nails may have increased the susceptibility of toe nails to get onychomycosis.

Considering the etiology of onychomycosis in this patient's sample, dermatophytes were the leading pathogen followed by NDM and *Candida* corroborating some previous findings [15, 17]. However, a previous Sri Lankan study using 128 onychomycosis patients, has reported that the prevalence of NDM (45.8%) was high, followed by yeasts (34.1%); and dermatophytes (20%) [11]. Furthermore, Chi et al. [20] in a group of 375 patients with onychomycosis have reported that there were 60.5% patients with dermatophytes, 31.5% patients with *Candida* and 8% patients with NDM infection. According to Jesudanam et al. [6], the predominant pathogens obtained from onychomycosis lesions in an Indian population were *Candida* (56.7%), followed by dermatophytes (38.2%) and NDM (3.37%). Hence, it is reasonable to conclude that the etiological agents of onychomycosis vary in different settings while dermatophytes, NDMs and *Candida* remain as common pathogens with variable rates of prevalence. Further investigations with a large population may be supportive to confirm the contribution of various pathogens causing onychomycosis in Sri Lankan patients.

Many investigators have claimed that *T. rubrum* was the main etiological agent for onychomycosis [12, 16, 19]. Extensive reviews have also pointed out that onychomycosis is primarily caused by dermatophyte, *T. rubrum* [4, 15]. According to the present study, the commonest dermatophytes isolated from the infected nails were *T. mentagrophytes* followed by *T. rubrum.* Supporting the above observations, some previous investigators have reported that the most commonly isolated dermatophyte was *T. mentagrophytes* with regard to onychomycosis [14]. Hence, further studies are necessary to ascertain the leading dermatophytes causing onychomycosis in local settings.

The second commonest cause for onychomycosis in these patients was NDMs and the leading pathogen was *Fusarium* species which has already been recognized as the commonest NDM associated with onychomycosis [21]. However, several other local and overseas studies have identified *Aspergillus* species as the predominant NDM responsible for onychomycosis [11 17, 22 - 24]. Ranawaka et al. [25] have reported that the prevalence of *Fusarium* onychomycosis was 6.25% (8/128) in a local patient sample. In extensive research using Sri Lankan onychomycosis patients, Ranawaka et al., [26] have demonstrated that NDM were the predominant fungi (68.2%), followed by *Candida* species (21.6%) and dermatophytes (10.1%). NDM causing onychomycosis consisted of *Aspergillus* species (75.1%) followed by *Fusarium* species (8.9%) and *Penicillium* species (4.95%) [26]. *Fusarium* species being the leading NDM associated with onychomycosis in this study indicates that *Fusarium* species is also an important etiological agent for onychomycosis, Further studies with broader sample size are recommended to confirm this hypothesis.

Candida associated onychomycosis accounted for 27% of our sample and remained the third prevalent cause. According to Chi et al. [20] *Candida* species was the second commonest etiological agent for onychomycosis in a group of patients from Thaiwan resulting in 31.5% of infections. Foster et al. [27] in a survey of superficial mycoses in USA during 1999 to 2002 noted that *Candida* species caused approximately 70% of onychomycoses. It has been reported that *Candida* is emerging as an important pathogen with regard to onychomycosis [3]. Further studies using increased sample size are necessary to ascertain the exact prevalence of *Candida* associated onychomycosis in Sri Lankans.

To conclude, mycologically proven onychomycosis was common in the adults especially, from 20-69 years of age and it was frequent among housewives, health care workers and farmers in the current sample. Onychomycosis frequently affected the toe nails. Etiology was mainly dermatophytes followed by NDMs and *Candida*. Common dermatophytes associated with onychomycosis were *T. mentagrophytes* and *T. rubrum*. *Fusarium* spp. was the leading NDM associated with onychomycosis in this cohort.

Author declaration
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Conflict of interest
Authors declare no conflict of interest.
Funding
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Ethics statement
Ethics approval was obtained from the Ethics Review Committee of the Medical Research Institute (MRI) of Sri Lanka (53/2014 on 13 /11 /2014). Informed written consent was obtained from the study participants.
Author contribution
JAMAJ- prepared the proposal, conducted data and sample collection, performed all the laboratory experiments, analyzed data and prepared the manuscript.
GRR - supervised laboratory investigations and provided intellectual input in the experimental protocol and manuscript writing.
AN - provided intellectual input in experimental protocol and manuscript writing.
DMM - performed clinical diagnosis and provided intellectual input in manuscript writing.
JAMSJ - supported manuscript writing and final editing.
All authors have seen and approved the final version of the manuscript.
Data availability
Data will be available on request from the corresponding author.

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