Research Article

Prevalence, aetiology and factors associated with onychomycosis among patients with diabetes at a Tertiary Care Centre in Galle, Sri Lanka

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Abstract

Introduction: Epidemiological data of onychomycosis, a common fungal nail infection found in diabetics, is scarce in Sri Lanka.

Objective: To determine the prevalence, aetiological agents and associated factors of onychomycosis among patients with clinically suspected diabetics in southern Sri Lanka.

Method: A cross-sectional study was done using a sample of 97 diabetic patients suspected of having onychomycosis in a tertiary diabetes care centre in Galle. Nail clippings were collected, and microscopic and cultural examinations were done. Both microscopic and culture positive cases were considered as having onychomycosis.

Results: The prevalence of onychomycosis was 18.6% (95% CI: 11.38%, 27.73%). Non-dermatophyte moulds (NDMs) (55.7%) and yeast (30.9%) were the dominant aetiological agents. The leading pathogens found among NDMs were *Aspergillus* sp. (35.1%) and *Fusarium* sp. (1.4%) and *Candida albicans* (8.2%). *Aspergillus niger* (20.6%) was the most frequently isolated *Aspergillus* sp. in the NDMs. Frequent use of detergents and walking bare foot were the significant risk factors of onychomycosis. Only 15.5% of the participants were aware of onychomycosis.

Conclusion: Onychomycosis is prevalent to a considerable level among diabetics. Regular dermatological investigations of nails and health education are needed to combat the disease. Large scale research is warranted to confirm these assertions.

Keywords: *Onychomycosis, Diabetes Mellitus, Sri Lanka, Risk Factors, Candida*

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Introduction

Onychomycosis is derived from the Greek words "onyx" and "mykes" which mean "nail" and "fungus". It is an infection that can affect any part of the nail unit.[1] Prolonged blood glucose elevation resulting from diabetes causes extensive nail thickening and eventually increases the probability of onychomycosis.[2] It is more common with toenails due to the slower growth rate making it easy for the fungus to establish infection.[3] Approximately, one-third of patients with diabetes suffer from onychomycosis but the prevalence of onychomycosis among diabetics in Sri Lanka is not well documented.[4]

Onychomycosis is caused by three types of aetiological agents: dermatophytes, non-dermatophyte moulds (NDMs) and yeasts.[1] Dermatophytes in particular produce enzymes which degrade keratin in the nail plate.[5] Non dermatophytes require damaged or compromised nails to establish the infection, indicating that they are mostly secondary agents.[6] Diagnosis of onychomycosis caused by dermatophytes is by the direct microscopic evidence and/or culture as they are primary pathogens.[7] However, diagnosis of onychomycosis by non-dermatophyte fungi such as *Aspergillus* sp. is more complex as they are also environmental contaminants. Therefore, presence of fungal elements in direct microscopy as well as culture positivity is essential for the diagnosis to confirm that the culture is not an environmental contaminant.[8]

Sociodemographic variables such as age, gender, occupation, educational status, and underlying conditions such as psoriasis, hyperhidrosis, lifestyle and practices including the use of public bathing facilities, and concurrent dermatophyte infections at other body sites can predispose diabetics to fungal nail infections.[1,3,9]

Typical onychomycosis clinically manifests as linear, single, or multiple white or yellow-brown bands and violaceous, green or black discoloration of the nail plate. Other clinical features include subungual hyperkeratosis, onycholysis, and onychauxis. Based on nail localisation and type of invasion, onychomycosis presents as four main subtypes which are distal and lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), proximal subungual onychomycosis (PSO), total dystrophic onychomycosis (TDO).[10]

Onychomycosis is a clinical and mycological diagnosis.[11] Mycological examination is the only method to diagnose the exact aetiology of onychomycosis which in turn helps to decide on therapeutic prescription as many other conditions might result in the same clinical presentation. Proper mycological diagnosis is recommended before initiating systemic antimycotic treatment which may have multiple adverse events and drug interactions. If identified at an early stage, nail fungal disease can easily be managed by taking necessary early preventable measures. Delayed treatment could also lead to the spread of infection to the whole nail plate as well as other nails making it more challenging to treat.[1] Proper foot care is an integral part of diabetic management which is further aggravated by peripheral vascular and neurological complications. Onychomycosis, beyond its symptomatic nature, leads to social embarrassment as patients conceal their nails, causing physical, psychological, and social issues.[12]

The tropical environment of Sri Lanka with a high level of humidity and dust facilitates the luxurious growth of fungi in the environment. These environmental conditions predispose to superficial fungal infections including onychomycosis.[13] This study aimed at identifying prevalence, aetiology and associated factors of onychomycosis among diabetic patients suspected of having onychomycosis who presented at a diabetic clinic in a tertiary care center in southern Sri Lanka.

Methods

A descriptive cross-sectional study was conducted among diabetic patients attending the diabetic and endocrine clinic at the Teaching Hospital Karapitiya (THK), Galle, Sri Lanka. The sample size of 97 was determined using the formula for descriptive studies[14], assuming a 50% prevalence of onychomycosis among diabetics and taking the margin of error as 10%. Specimen processing was done at the mycology laboratory of the Faculty of Medicine (FOM), University of Ruhuna, Galle. Diabetic patients aged over 18 who gave consent were included. Exclusions were pregnant mothers with gestational diabetes and those unable to understand Sinhala or English.

An investigator-administered questionnaire was pre-tested with diabetic patients attending the University Medical Clinic at THK to ensure clarity and understanding of the questions. Informed written consent was obtained from the participants. Specimen collection was done in an enclosed area assuring privacy for participants after ensuring that they were off antifungal therapy for at least 2 weeks. The affected nail was cleaned with 70% alcohol and the superficial part of the nail which is likely to contain environmental fungal spores were scraped off. The affected part of the nail was then scraped, and subungual debris collected using sterile scalpel blades and forceps. Samples were collected onto separate black coloured paper pockets labeled with specific laboratory numbers and were processed at the mycology laboratory for analysis.

Microscopy: Nail clippings were examined for the presence of fungal elements under a light microscope after digesting with 30% potassium hydroxide (KOH) for 15 minutes.[15]

Culture: Another portion of each specimen was inoculated in duplicate on Sabouraud Dextrose Agar (SDA) with chloramphenicol with and without cycloheximide. The addition of cycloheximide to the media inhibits saprophytic fungi enabling isolation of slow-growing dermatophytes while chloramphenicol inhibits bacterial growth. The inoculated plates were incubated at 26 ^{0}C for up to four weeks and regularly inspected for visible fungal growth.[15]

Identification: Macroscopic identification of fungal cultures and tease mounts with lacto-phenol cotton blue (LPCB) staining facilitated morphological fungal identification. The slide culture technique was employed for species identification of mycelial fungi. Samples that had no growth after four weeks were reported as negative. [15,16,17]

Identification of candida species was accomplished by evaluating the morphological characteristics on rice agar, as well as the colony appearance and chromogenic properties on CHROMagar. [15,16,17]

Onychomycosis cases were defined as those with positive microscopic and culture results. Patients received routine laboratory reports by post, and those with evidence of onychomycosis were referred to a dermatologist for further treatment.

For data analysis, IBM Statistical Package for the Social Sciences (SPSS) version 26 was utilised. Frequency tables and cross tables were generated. The mean values of continuous variables were reported with standard deviations. The Chi-square test was used to compare categorical data. Statistical significance was set at $p<0.05$.

Results

Nail clippings were collected from 97 participants, with 76 females and 21 males. The mean age was 61 years (SD = 11). Details of the participants are given in Table 1.

Table 1: Sociodemographic features of the study participants (n=97)

Variable	Number of participants	Percentage %
Age group (years)		
≤55	22	22.7
>55	75	77.8
Mean ± SD (IQR)	60.99 ± 10.93	
Gender		
Male	21	21.6
Female	76	78.4
BMI classification (kg/m^2)		
Underweight	5	5.2
Normal	56	57.7
Overweight	35	36.1
Obese	1	1.0
Mean ± SD (IQR)	24.22 ± 3.79	
Occupation		
Retired government officers	25	25.8
Tea pluckers	12	12.4
Agriculture & Farming	9	9.3
Housework	51	52.5
Educational status		
Uneducated	10	10.3
Primary	33	34.0
Secondary	42	43.3
Graduate	12	12.4
Diabetes type		
Type 1	2	2.1
Type 2	95	97.9
Diabetes duration (years)		
1-10	65	67.0
>10	32	33.0
Diabetes status based on HbA1c level		
≤6.4%	24	24.8
>6.5%	73	75.2
Mean ± SD (range)	7.68 ± 1.58	

Only 3.1% of participants reported wearing protective boots and gloves during gardening. About 57.7% were walking barefoot, contrary to the advice given at the clinic. Only 11.3% used tight shoes, and 54.6% exhibited poor nail grooming. Although 9.3% of males had a history of smoking none currently smoked. Frequent detergent usage and hand immersion were reported in the noticeably high proportions of 66.0% and 69.1% respectively. Prior antifungal treatment was taken by 16.5%, with 4.1% still on occasional self-medication. Fungal infections other than onychomycosis were noted in 13.3%, with 8.2% having toe web intertrigo, while 1.0% had tinea capitis and tinea cruris. The remaining participants (3.1%) were diagnosed as having pityriasis versicolor based on their history and clinical diagnosis of previous infections.

The majority of patients (81.4%) had only toenail involvement with 4.1% having only fingernail involvement and 14.4% having both. Among these patients, 88.7% showed an involvement of the first toenail.

Based on nail appearance, the collected nail samples were categorised into the onychomycosis subtypes. DLSO was dominant (51.6%) with TDO the second commonest (28.9%), followed by WSO (7.2%) and PSO (2.1%). Of the 97 diabetic patients clinically suspected of having onychomycosis, only 18 (18.6%) (95% CI: 11.38%, 27.73%) were confirmed with fungal infection by direct microscopy and culture.

Aspergillus sp. (35.1%) was the most frequently isolated non dermatophyte mould. Among the isolated *Aspergillus* sp., *Aspergillus niger* was the commonest (20.6%). *C. albicans* (8.2%) and *C. tropicalis* (7.2%) were the most commonly isolated yeasts (Table 2).

Table 2: Fungal species isolated from nail specimens

Aetiology		Frequency	Percent %
Culture positive		**84**	**86.6**
	Direct microscopy positive	18	18.6
	Direct microscopy negative	66	68.0
Dermatophytes		**0**	**0**
Non dermatophytes		**54**	**55.7**
	Aspergillus sp.	34	35.1
	Fusarium sp.	14	14.4
	Neoscytalidium sp.	3	3.1
	Scedosporium sp.	2	2.1
	Acremonium sp.	1	1.0
Yeasts		**30**	**30.9**
	Candida albicans	8	8.2
	Candida tropicalis	7	7.2
	Candida glabrata	2	2.1
	Candida parapsilosis	1	1.0
	Other *Candida*	12	12.4
Culture negative		**13**	**13.4**
	Direct microscopy positive	0	0
	Direct microscopy negative	13	13.4
Total		**97**	**100**

A few patients (15.5%) were aware of onychomycosis with the rest (84.5%) having never known or heard of onychomycosis, even though they were health-educated regarding proper nail grooming.

No significant differences were observed between patients with onychomycosis and the rest of the study participants with respect to age, gender, level of education and BMI (Table 3). Medical conditions such as diabetic neuropathy, history of antifungal medication, family history of nail diseases and duration of diabetes and control of diabetes (Hb1Ac < 6.4) were not significantly associated with onychomycosis. Among habits and practices that were investigated, frequent detergent use (p=0.02) and walking barefoot (p=0.01) were significant predictors for development of onychomycosis. None of the other habits and practices that were investigated, including nail grooming and frequent immersion of hands in water showed any significant association with developing onychomycosis. However, it was noted that a considerably large proportion of those who had history of antifungal medication (31.3% versus 16.1%), family history of nail diseases (33.3% versus 16.5%), frequent immersion of hands in

water (22.4% versus 10.0%) and uncontrolled diabetes (21.3% versus 9.1%) had onychomycosis compared to that of others.

Table 3: Risk factors for onychomycosis among study participants (n=97)

Risk Factors		Positive cases (n= 18)		Negative cases (n=79)		P value
		No:	Frequency (%)	No:	Frequency (%)	
Age	≤55 years >55 years	3 15	13.64 20.00	19 60	86.36 80.00	0.49
Gender	male female	3 15	14.29 19.74	18 61	85.71 80.26	0.56
Educational level	Primary or less Secondary or above	8 10	18.60 18.52	35 44	81.40 81.48	0.99
BMI	Normal or underweight Overweight	12 6	19.35 17.14	50 29	80.65 82.86	0.78
Diabetes Duration	≤10 years >10 years	12 6	18.46 18.75	53 26	81.54 81.25	0.97
HbA1c	≤6.5% >6.5%	2 16	9.09 21.33	20 59	90.91 78.67	0.19
Diabetes neuropathy	yes no	8 10	21.05 16.95	30 49	78.95 83.05	0.61
Family history of nail disease	yes no	4 14	33.33 16.47	8 71	66.67 83.53	0.15
Frequent detergent use	yes no	16 2	25.00 6.06	48 31	75.00 93.94	0.02
Walk barefoot	yes no	15 3	26.79 7.32	41 38	73.21 92.68	0.01
History of antifungal medication	yes no	5 13	31.25 16.05	11 68	68.75 83.95	0.15
Poor nail grooming	yes no	8 10	15.09 22.73	45 34	84.91 77.27	0.33
Frequent immersion of hands in water	yes no	15 3	22.39 10.00	52 27	77.61 90.00	0.14

*P<0.05 is considered as significant.

Discussion

Prevalence of onychomycosis among diabetics who were suspected of having onychomycosis was 18.7% in this study. A similar study done in the Colombo South Teaching Hospital in 2015 found a prevalence of 85%. However, this study only included patients who were culture positive without microscopic evidence of fungal elements for the diagnosis of onychomycosis.[4] The low prevalence of onychomycosis in our study in contrast to this study could be due to the inclusion of direct microscopic results as well as culture positivity for diagnosis. As the non-dermatophyte fungi such as aspergilli are environmental contaminants, the presence of microscopic evidence of fungal infection is essential for the diagnosis of non-dermatophyte infections. The prevalence of onychomycosis in patients with diabetes was 86.6% in our study

if only culture positives were included without considering the direct microscopic results for diagnosis.

Previous studies conducted in other countries have indicated that the prevalence of onychomycosis among diabetics varied from 17% to 34%.[11,18,19] The prevalence of onychomycosis among diabetics varies widely across countries as well as across timelines in the same country. In our study, it was observed during the data collection period that the majority of the patients who attended the diabetes clinic had paid considerable attention to foot care as most of them presented at the clinics with clean and well-groomed nails. This may be attributed to proper health education on foot care at the diabetic clinics in this tertiary care centre in Galle leading to lower rates of onychomycosis among the diabetics.

In this study, *Aspergillus* sp. (35.1%) and *Candida* sp. (30.9%) were identified as the primary causes of onychomycosis among diabetic patients in Galle, Sri Lanka. Similar prevalence rates of NDMs (45.8%) dominated by *Aspergillus niger* and yeasts (34.1%) were reported in previous Sri Lankan studies.[4,20] *Aspergillus* sp. is identified as an emerging pathogen in diabetic patients with onychomycosis in Sri Lanka.[4] In contrast, another local study which involved a randomly selected mixed population comprising both diabetic and non-diabetic patients clinically diagnosed with onychomycosis, reported dermatophytes (40%) as the primary aetiology, followed by NDMs (33%) and yeasts (27%).[21] A multicentre study specifically targeting toenail onychomycosis in diabetics in temperate regions found dermatophytes to be the predominant pathogen, followed by non-dermatophyte mould (NDMs).[18] However, this trend was not observed in the current study which may be due to climatic changes contributing to a unique difference in the study population in southern Sri Lanka.

In the current study, *Candida* sp. was isolated following *Aspergillus* sp. This prevalence of candida among diabetic patients contrasts with previous studies conducted in Sri Lanka.[4,21,22] *Candida albicans* has been reported as the predominant organism causing onychomycosis among diabetics, with prevalence rates of 60.9%[23] and 48.1%[24] in some countries, which was considerably higher than the findings in the current study. This study reveals a distinctive pattern of aetiology, characterised by a higher prevalence of *Aspergillus* sp. and *Candida* sp. and absence of dermatophytes in onychomycosis observed in Galle, contrasting with previous studies.[2,11]

Studies[9,24,25] have found gender, age, family history of nail diseases, use of antifungal medication and the degree of glycaemic control as factors associated with onychomycosis in diabetic patients. However, our results did not find such associations and further studies with large sample sizes are needed to identify such associations.

In this study, detergent use (p=0.02) and walking barefoot (p=0.01) exhibited a significant association with the development of onychomycosis among diabetics. However, no significant association was identified between the duration of daily work hours and superficial fungal infections[13] in a Sri Lankan study on superficial fungal infections among janitors, despite frequent exposure to water, detergents, and cleansers. Although the evidence is weak, it is advisable to educate diabetic housewives who tend to use detergents frequently on preventive measures to minimize the risk of developing onychomycosis.

A previous Sri Lankan study also noted that rubber boots or closed shoes are seldom used by farmers and labourers in muddy environments and in tea plantations.[20] As there is a significant association between walking barefoot and the development of onychomycosis shown in the

current study, measures to enhance compliance with the appropriate use of footwear in the Sri Lankan population is timely.

In our study, DLSO (51.6%) was found to be the commonest type of onychomycosis followed by TDO, WSO, and PSO. A previous Sri Lankan study reported DLSO in 76% of their participants, also noting that the primary site of entry for invading organisms was most often the distal and lateral margins.[20] Another study has demonstrated that DLSO typically affects the first toe.[18] Similarly, the current study also found that the first toenail was the most frequently affected nail type (88.7%) having DLSO.

The awareness of patients with onychomycosis was very poor and only 15.5% had heard or known about the risk of onychomycosis in diabetes, even though nail grooming was done for most clinic patients. A similar study conducted in Sri Lanka on superficial fungal infections demonstrated an unsatisfactory level of knowledge (42%) among the study population.[13] Although patients are instructed on the proper healthy habits of nail grooming and foot care in many regions in Sri Lanka, they are unaware it is done for the prevention of onychomycosis. It is important to disseminate the facts and figures about onychomycosis among diabetic patients as a part of clinical services to enhance their healthy practices.

The study faced certain limitations, including challenges in specimen collection, as patients intentionally cut their toenails and fingernails following clinic advice. Some participants had used antifungals, complicating sample collection. Time constraints hindered sample repetition for culture-positive patients to confirm the reliability of the tests done.

Conclusion

The prevalence of onychomycosis in this target population of diabetic patients was found to be 18.7% indicating that a smaller proportion of diabetics than expected suffer from onychomycosis. However, prevention and control strategies should be strengthened as the consequences can be significant in diabetes. NDMs, particularly *Aspergillus* sp. (35.1%), were the predominant aetiological agents, followed by yeasts (30.9%), with *Candida albicans* accounting for 8.2%. Based on the current study and previous studies conducted in Sri Lanka, the aetiological pattern of onychomycosis in Sri Lanka is different from other geographical regions as it is characterised by a higher prevalence of *Aspergillus* and *Candida* compared to dermatophytes. Walking barefoot and frequent detergent use should be discouraged among diabetics in Sri Lanka. Despite limited awareness among diabetics about onychomycosis, the disease prevalence is low indicating the success of good health education regarding proper nail care among the risk population.

Declaration

Conflicts of Interest: The authors of this manuscript declare that they have no conflicts of interest that could influence the objectivity or integrity of the research presented in this paper.
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(OOUTH/HREC/463/2021AP) granted ethical approval for this study. In line with the standards for human experimentation and the revised 2000 Helsinki Declaration, all participants willingly provided informed consent. To ensure their consent was obtained, all recruited participants were required to complete informed consent forms.
Statement on author contributions:
JGMA. Thilakarathna gathered data, processed samples, analysed and formulated the manuscript.
HHPMJ. Thabrew supervised the project and edited the manuscript.
B. Perera assisted in statistics and data analysis.
HKTA. Gunasekara supported the laboratory work.

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