

# Proportion of lower limb fungal foot infections in patients with type 2 diabetes at a tertiary care hospital in Sri Lanka

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### ABSTRACT

**Background:** Superficial fungal foot infection (SFFI) in diabetic patients increases the risk of developing diabetic foot syndrome. Sixteen percent of urban population is suffering from diabetes in Sri Lanka. As the diabetes patients are more prone to get fungal foot infections, early intervention is advisable owing to the progressive nature of the infection. There is no data on the prevalence of SFFIs in diabetic patients in Sri Lanka. **Objective:** To determine the etiological agents causing SFFI in patients with type 2 diabetes. **Materials and Methods:** Three hundred eighty five diabetic patients were included. Nail clippings and swabs were collected from the infected sites using the standard protocol. Laboratory identification was done and pathogens were identified to the species level by morpho physiological methods. **Results:** Clinically 295 patients showed SFFI, of which 255 (86%) were mycologically confirmed for infection. Out of 236 direct microscopy (KOH) positives, 227 (96%) were culture positive. Two hundred and fifty one patients (98%) with SFFI had diabetes for more than 10 years. Of the patients with SFFIs 92% had >100 mg/dl FBS and 81% had >140 mg/dl PPBS levels and 80% had both elevated FBS and PPBS. Non-dermatophyte fungal species were the commonest pathogens followed by yeast and dermatophytes. **Conclusion:** *Aspergillus niger* was the commonest pathogen followed by *Candida albicans*. SFFIs were seen significantly with the increasing age, gender, duration of diabetes and with less controlled glycaemic level.

**Key words:** Diabetes, fungal foot infections, superficial mycosis

## INTRODUCTION

Superficial fungal foot infections (SFFI) in diabetes increase the risk of developing diabetes foot syndrome which is serious sequelae.<sup>[1]</sup> Higher percentage (85%) of patients with prolong diabetes suffer from SFFIs<sup>[2]</sup> which is a risk factor for lower limb cellulites.<sup>[3]</sup> Infected nails, serve as a reservoir for recurrent infection.<sup>[4]</sup> Early intervention is advisable owing to the progressive nature of the fungal infection. As there is

no documented data on the prevalence of SFFIs in diabetes in Sri Lanka, we investigated the prevalence of fungal foot infections among diabetic patients in a tertiary care setting.

## MATERIALS AND METHODS

### Ethics

Ethical clearance was obtained from the ethical committee of University of Sri Jayewardenepura, Sri Lanka (Human biology/2012/02) and from the ethical committee of Colombo South Teaching Hospital, Sri Lanka (228).

### Study design

A descriptive cross sectional study conducted over a period of one year.

### Selection and distribution of participants

Three hundred and eighty five type 2 diabetic patients,

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who were suffering from diabetes for more than 5 years and attending the diabetic clinic at a Tertiary care hospital, were included in the study after obtaining their informed written consent. Pregnant mothers, debilitated patients who suffered from diabetes for less than five years were excluded. One hundred and thirty patients were males and 255 were women.

An interview based questionnaire was filled after getting the informed written consent. In addition to demographic data, patients were asked questions on knowledge and practices about the fungal foot infections, its risk and preventive measures. Sampling was done after obtaining informed written consent of the patients who volunteered for the research. Clinical examinations of patients' toe nails or other infected sites in the foot were performed by a clinical microbiologist. The affected areas were cleaned with 70% alcohol and sterile scissors, nail-cutters were used to cut the infected part of the toe nail. The nail clippings were collected to black papers (5" × 5"). Swabs were collected from the infected toe-webs and soles of the patients and were inoculated on Sabourand's Dextrose Agar (SDA) (Oxoid, USA) plates directly. Specimens were sent to the Department of Microbiology for laboratory diagnosis. Nail clippings were analyzed by direct microscopy by using 10% KOH (Potassium hydroxide).<sup>[5]</sup> Gram stain was done for all the swabs. Subsequently, nail clippings and swabs of each patient were inoculated on SDA (Oxoid, USA) plates with and without antimicrobials (chloramphenicol (50 µg/ml) and cycloheximide (100 µg/ml) and incubated at 25°C- 30°C aerobically for 3 weeks. Filamentous fungi were identified by colony morphology and slide culture. Yeast species were identified by means of Gram's stain, germ tube test and carbohydrate assimilation test. For the germ tube test, 24 hour old pure yeast cultures were used. Yeast colonies were inoculated to 0.5 ml of horse serum and incubated at 37°C for 2 1/2 hours and looked for the production of germ tube. Carbohydrate Assimilation Test – Commercially (Difco Laboratories) available Yeast Nitrogen Base (YNB; carbon free) agar dissolved in distilled water was autoclaved in 15 ml volumes within universal containers. The containers were allowed to cool to 45°C in a water bath. Heavy suspensions of the yeasts to be identified were prepared using one loopful of the yeast isolate in 5 ml of sterile distilled water and 5 drops from each suspension were added to a separate YNB agar container at 45°C. Each seeded agar container was poured into a 90 mm sterile Petri dish and allowed to set at room temperature. Few crystals of the carbohydrates Glucose, Maltose, Sucrose, Lactose, Galactose, Cellibiose, Raffinose, Inositol, Xylose, Trehalose and Melibiose were carefully placed on the surface of the seeded YNB agar (Difco

Laboratories) plate according to a template and incubated at room temperature (30°C) up to 48 hours. Enhanced growth of the yeast at the points where the sugars were placed was detected visually and indicated assimilation of the particular sugar and when the sugar was not utilized there was no visible growth of the yeast. Statistical analysis - Laboratory investigations and data from the questionnaires were entered into Microsoft office Excel. Statistical analysis, more specifically odd ratio and Chi-square tests were performed using the software package, SPSS version 15. All inferential statistics were tested at 95% level of confidence.

## RESULTS

Three hundred and eighty five, type 2 diabetic patients who attended the diabetic clinic at a Tertiary care hospital were included in the study. Amongst them, 255 were women with a mean age of 56.2 years (Standard deviation (SD 11.27) and 130 were men with a mean age of 57.8 years (SD 11.76). All the patients were clinically examined by a clinical microbiologist for superficial fungal foot infections (SFFIs). Among the 385 diabetic patients, 295 (77%) were clinically suspected to have SFFI. Among the clinically suspected patients, 203 (69%) were women with a mean age of 57.5 years (SD 10.88) and 92 (31%) were men, with a mean age of 59.9 years (SD 11.57). Among the 295 patients who were clinically suspected, 255 (86.4%) were mycologically confirmed to have fungal infection. The Tables 1 and 2 shows the proportion of the SFFIs by fungal culture and KOH results.

Among the 236 samples, which were positive for direct microscopy technique, 227 (96.2%) were culture positive. The false negativity of direct microscopy was 3.8%, as 9 samples which were positive for direct microscopy showed negative culture results. Table 3 shows the identification of SFFIs with related to the duration of diabetes.

**Table 1: Proportion of SFFIs by culture among gender**

	Culture (%)		Total (%)
	Positive	Negative	
Sex			
Female	170 (83.7)	33 (16.3)	203 (100.0)
Male	85 (92.4)	7 (7.6)	92 (100.0)
Total	255 (86.4)	40 (13.6)	295 (100.0)

P value - 0.044, SFFIs: Superficial fungal foot infections

**Table 2: Results of direct microscopy (KOH) and culture**

Direct microscopy	Culture positive (%)	Culture negative (%)	Total (%)
Positive	227 (96.2)	9 (3.8)	236 (100.0)
Negative	28 (47.5)	31 (52.5)	59 (100.0)
Total	255 (86.4)	40 (13.6)	295 (100.0)

P value - 0.000, KOH: Potassium hydroxide

Among the clinically suspected, 50 patients with positive culture results were less than 40 years of age. Two hundred and five patients with positive culture results were more than 40 years of age. Since the *P* value was 0.011 (<0.05), occurrence of SFFI according to culture was found to be statistically significant with increasing age. Table 4 explains the proportion of SFFIs with the fasting blood sugar (FBS) and post prandial blood sugar levels (PPBS). Since the *P* value was 0.015, occurrence of SFFI was found to be statistically significant according to culture with less controlled FBS levels. Since the *P* value was 0.003, occurrence of SFFI was found to be statistically significant with less controlled PPBS levels.

Among the study population 142 (37%) of patients assumed they had fungal infections, but 295 (77%) were clinically

**Table 3: Proportion of SFFI with the duration of diabetes**

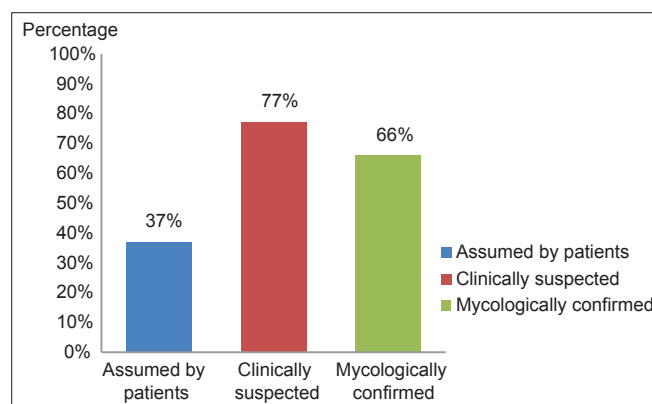
Duration	Culture (%)		Total (%)
	Positive	Negative	
5 - 10 years	4 (40.0)	6 (60.0)	10 (100.0)
11-15 years	96 (88.9)	12 (11.1)	108 (100.0)
>15 years	155 (87.6)	22 (12.4)	177 (100.0)
Total	255 (86.4)	40 (13.6)	295 (100.0)

*P* value - 0.000, SFFI: Superficial fungal foot infections

**Table 4: Proportion of SFFI with FBS and PPBS levels**

FBS levels	Culture (%)		Total (%)
	Positive	Negative	
<99 mg/dl	20 (57.1)	15 (42.9)	35 (100.0)
>100 mg/dl	235 (90.4)	25 (9.6)	260 (100.0)
Total	255 (86.4)	40 (13.6)	295 (100.0)
PPBS level	Culture (%)		Total (%)
	Positive	Negative	
<139 mg/dl	48 (75.0)	16 (25.0)	64 (100.0)
>140 mg/dl	207 (89.6)	24 (10.4)	231 (100.0)
Total	255 (86.4)	40 (13.6)	295 (100.0)

FBS: Fasting blood sugar, SFFI: Superficial fungal foot infections, PPBS: Post prandial blood sugar



**Figure 1:** The relationship between the patient assessment on SFFIs, clinical finding and mycological results

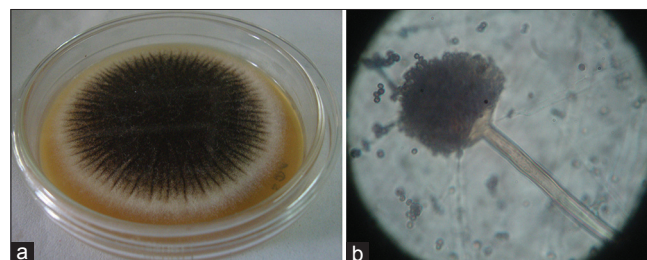
suspected and 255 (66%) were mycologically confirmed [Figure 1]. It is evident that the actual frequency of fungal infections was underestimated by the patients. In the questionnaire all the patients said that they examined their feet every day for infections and injuries. Also, all of them are cutting their nails regularly and 265 (69%) of the patients dry their feet after a bath. Among all the patients 150 (39%) used skin cream or oil for foot care. Herbal medication was used by 13 (3.4%) of patients for foot infections. Among the study population, 150 (39%) patients had suffered from SFFI previously. All of them had used anti fungal ointments as a treatment for the infection. Out of these patients 110 (73%) showed positive culture results indicating a recurrence of SFFI. According to the nail characteristics, 283 (73%) had hard or brittle nails, 274 (71%) had discolored nails, and 98 (26%) had dry rough feet. According to regular activities, 206 (53%) of patients undertook activities associated with water and 126 (33%) attended on agricultural activities.

### Results of laboratory identifications

Out of the 482 organisms which were isolated from the collected specimens, 349 (72%) were non dermatophyte fungal species, 78 (16%) were yeast species and 55 (12%) were dermatophyte fungal species. *Aspergillus niger* [Figure 2a and b] was predominant in nail infections followed by *Fusarium.spp* [Figure 3a and b], *Candida albicans* and *Aspergillus flavus*. In skin mycosis *Candida albicans* was predominant followed by *Aspergillus terreus*, *Trichoderma.spp* [Figure 4a and b] and *Trichophyton tonsurans* [Figure 5a and b]. Table 5 shows the different fungal species isolated from the specimens, while Table 6 shows some of the fungal species isolated from the lesions.

## DISCUSSION

Diabetic patients represent a unique group of individuals who are more prone to develop infections than others. A broader range of etiological agents from primary pathogens to opportunistic fungal species is one of the characteristic of fungal infections combined with diabetes. The growing diabetic population in Asia<sup>[6]</sup> and the high frequency of fungal foot infections<sup>[7]</sup> in patients with diabetes present a considerable health problem. The clinical



**Figure 2:** (a and b) *Aspergillus niger*

presentations of fungal infections are unpredictable and poor, often leading to delayed diagnosis.<sup>[8]</sup> Our study assessed the prevalence of lower limb fungal foot infections in patients with type 2 diabetes at a Tertiary care hospital in Sri Lanka.

In our study, among the 385 diabetic patients, 66% were mycologically confirmed to have Superficial Fungal Foot Infections (SFFI). Non dermatophyte fungal species were the most common pathogen causing SFFI, followed by yeast and dermatophyte fungal species. Nail infections was the commonest type of SFFI among the study population which is in agreement with the previous study done by Yehia *et al.*<sup>[9]</sup> Among the patients who were clinically suspected to have SFFI, 14% showed negative culture results, even though they had been clinically suspected to have SFFI. These patients were using anti fungal ointments at the time of collection and therefore the growth of the fungal species might have been inhibited. *Aspergillus niger* was the most common causative pathogen which caused nail infections followed by *Fusarium.spp* and *Candida albicans*. In a previous study, *Aspergillus niger* was found to be the commonest cause of superficial fungal infections, among the cleaners.<sup>[10]</sup> Although, these data cannot be directly compared due to the two different populations this may have some association with the region. Our results were also in accordance with previous investigations of Eckhard *et al.*,<sup>[11]</sup> Nair *et al.*,<sup>[12]</sup> where they have also found *Aspergillus niger* as the commonest cause of fungal foot infections in diabetes.

In contrast to our study, Gupta *et al.*,<sup>[13]</sup> Romano *et al.*,<sup>[14]</sup> have reported that dermatophyte fungal species such as *Trichophyton.spp*, *Microsporum.spp* and *Epidermophyton.spp* were more common in toe nail infections in diabetic foot. This conflicting data may be because of the climatic factors in different geographical areas in the world and differences in habits, cultures among different nations and also may be due to the emerging pathogens.

Finding of *Aspergillus niger* as the commonest pathogen with a high proportion (43%) causing superficial fungal infection is a significant finding not only locally but globally, as this organism is known as a saprophytic organism found in soil.<sup>[15]</sup> In South East Asia the hot, humid climate may have contributed to the changing etiologic pattern in SFFIs. Further it is a known fact that many individuals walk bare foot and do not pay enough attention to foot care which is especially important in diabetic foot. This is an alarming finding especially in the diabetics and may well be an emerging pathogen globally. On the other hand misuse of antifungal ointments without prescription may have contributed to this new emerging pathogen. This finding

**Table 5: Spectrum of fungal pathogens isolated from the lesions**

Etiological agents	Total number of isolates	In nail infection	In skin mycosis
<i>Aspergillus niger</i>	208	203	5
<i>Candida albicans</i>	53	29	24
<i>Fusarium.spp.</i>	40	37	3
<i>Aspergillus terreus</i>	24	14	10
<i>Aspergillus flavus</i>	21	21	0
<i>Trichoderma.spp</i>	19	13	6
<i>Trichophyton schoenleini</i>	17	17	0
<i>Trichophyton verrucosum</i>	14	11	3
<i>Aspergillus fumigatus</i>	11	8	3
<i>Candida parapsilosis</i>	9	4	5
<i>Trichophyton tonsurans</i>	8	3	5
<i>Dematecius.spp.</i>	8	7	1
<i>Microsporum gypseum</i>	7	5	2
<i>Nigrospora.spp</i>	7	7	0
<i>Mucor.spp.</i>	7	7	0
<i>Epidermophyton floccosum</i>	5	3	2
<i>Candida tropicalis</i>	5	4	1
<i>Trichophyton mentagrophyton</i>	4	3	1
<i>Penicillin.spp</i>	4	4	0
<i>Candida ketyl</i>	4	2	2
<i>Candida lusitaniae</i>	3	3	0
<i>Candida guilliermondii</i>	2	2	0
<i>Candida glabrata</i>	1	1	0
<i>Cryptococcus.spp</i>	1	1	0

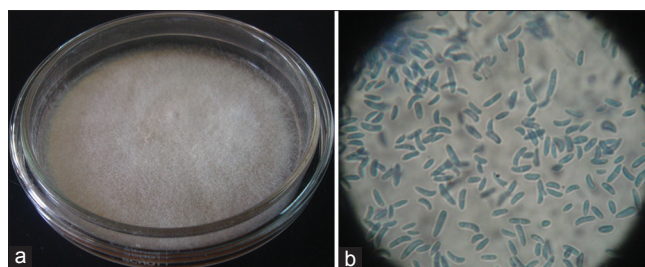
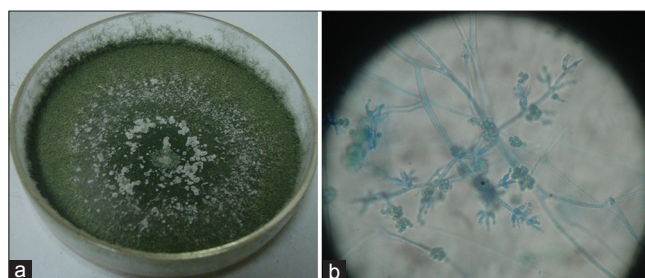
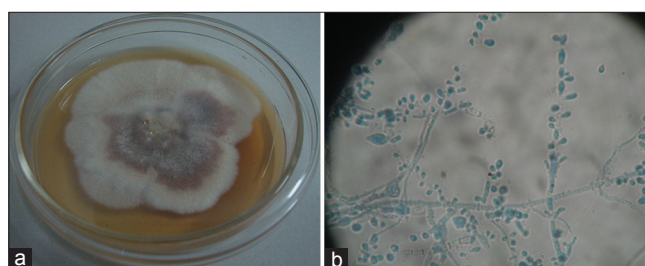
has to be investigated in different geographic populations globally.

This study is restricted to a Tertiary Care Hospital in Colombo which contains more than >1000 beds and is one of the main Teaching hospitals in Sri Lanka. There is no documented data available on the prevalence of superficial fungal infections in diabetics in Sri Lanka. Hence, our findings cannot be directly compared or applied to the whole Sri Lankan population. A study done for a period of 5 years (1974-1980) had found dermatophytes (*Trichophyton rubrum*) as the most prevalent causative agent for superficial fungal infections in the country.<sup>[16]</sup> In another study, which looked at the pattern of the superficial fungal infections in two time periods (1974-78 and 1990-94) have found increased *Tenia pedis* infection in 1990-94 period compared to 1974-78, which the author thinks is a result of increased habit of wearing shoes.<sup>[17]</sup> This result is in contrast to the findings where we found *Aspergillus niger* as a commonest causative agent. Further our findings of *Aspergillus niger* as the true pathogen is consolidated as 60% of the clinical specimens gave both direct smear and culture positivity for this organism. Our results and other reports including Nair *et al.*,<sup>[12]</sup> Fatar *et al.*,<sup>[18]</sup> showed that *Candida albicans* was the most common fungal pathogen isolated from skin lesions in the lower limb, followed by non *albicans* species. Among the non *albicans* species, *Candida Parapsilosis* was the commonest causative

**Table 6: Fungal species isolated from the lesions**

Name of the fungal agent	Culture morphology	Microscopic view
<i>Aspergillus niger</i>	Figure 2a  Septate, hyaline hyphae. Colonies consist of a compact white basal felt covered by a dense layer of dark-brown to black conidial heads in radial grooves. Reverse is buff color	Figure 2b  Magnification - x40 Stain- Lacto phenol cotton blue. Conidial heads are dark brown to black, radiate with metulae twice as long as the phialides. Conidia brown and rough walled
<i>Fusarium spp</i>	Figure 3a  Colonies are usually fast growing. Mycelia are fluffy, cottony, and range in color from white to pale violet. Reverse is buff or yellow in color	Figure 3b  Magnification - x40 Stain- Lacto phenol cotton blue. Macroconidia are fusiform- to sickle-shaped, Microconidia are 1- to 2-celled, hyaline, pyriform, fusiform or curved.
<i>Trichoderma spp</i>	Figure 4a  Colonies are fast growing, at first white and downy, later developing yellowish-green to deep green compact tufts, in concentric ring-like zones on the agar surface	Figure 4b  Magnification - x40 Stain- Lacto phenol cotton blue. Conidiophores are repeatedly branched, bearing clusters of divergent, flask-shaped phialides. Conidia are green with smooth walls and are formed in conidial heads, clustered at the tips of the phialides
<i>T. tonsurans</i>	Figure 5a Surface is velvety or powdery, flat with a raised centre. Color may vary from yellow, rose to brownish. The reverse is yellow to reddish-brown	Figure 5b Magnification - x40 Stain- Lacto phenol cotton blue. Septate hyphae micro conidia varying from long clavate to broad pyriform are at right angles to the hyphae. Smooth, thin-walled, irregular, clavate macro conidia are present

agent followed by *Candida tropicalis* and *Candida lusitanae*. Similar, results were reported in Missoni *et al.*,<sup>[19]</sup> and Missoni *et al.*<sup>[20]</sup> Risk factors for *Candida parapsilosis* infection include previous traumatic dystrophy of the foot and exposure to soil during activities such as gardening. Some yeast species like *Candida albicans*, *C. parapsilosis* and *C. guilliermondii* constitute the physiologic flora of the feet.<sup>[21]</sup> They can shift to an invading

**Figure 3:** (a and b) *Fusarium spp***Figure 4:** (a and b) *Trichoderma spp***Figure 5:** (a and b) *T. tonsurans*

mycelia fungal form due to the nourishment given by the hyperglycemic state in diabetic patients to cause *Candida* infections in lower limbs.<sup>[22]</sup> In our study, Cryptococcal species was identified in one patient. Cryptococcosis is a life threatening opportunistic fungal infection.<sup>[23]</sup> As diabetic patients are immune compromised, they are at high risk of developing the infection when they are exposed to soil contaminated with bird droppings during activities such as gardening and walking bare foot.

Fungal culture results were found to be highly sensitive over the KOH results. According to the Chi-square test, the *P* value was 0.000 (<0.05). Previous studies have shown that KOH false negativity was 5-15%.<sup>[24]</sup> In our study, as 3.8% showed positive results in direct microscopy and negative results in culture, KOH false negativity was less than 15%. These results could be due to lack of viable fungal filaments in the sample due to the use of anti fungal ointments. Technical errors such as use of less concentrated KOH and not adequate incubated time can also give false negative KOH result.

Even though majority of the study population who were clinically suspected to have SFFI were females, the percentage of culture positive was more in specimens collected from males. Occurrence of SFFI was found to be statistically significant with male gender (Chi-square test,  $P$  value was 0.044). Fata *et al.*,<sup>[18]</sup> reported that gender related factors such as differences in life style, professional activities, and sports activities can affect the skin and nail structure of the foot. Therefore, the occurrence of SFFI might differ among males and females.

The statistical analysis of the study using odds ratio with 95% confidence interval showed that, higher number (88%) of patients were reported to have suffered from diabetes for more than ten years. In concordance with Chi-square the  $P$  value was 0.000. Therefore prevalence of SFFI was significantly associated with the increased duration of diabetes. Similar results were reported in Al-Mutairi *et al.*,<sup>[25]</sup>

Also, the presence of SFFI was found to be statistically significant with the advancing age of the diabetic patient ( $P$  value was 0.011). The increase in the prevalence of fungal foot infections with advancing age has been reported in previous investigations.<sup>[2,7,13]</sup> Koklu *et al.*,<sup>[26]</sup> reported that the diabetic patients more than 50 years are more susceptible to SFFI because the increased thickness of the nail plate and decreased growth rate of the nail. Accordingly prolong diabetes and older age can lead to SFFI because these patients provide a favorable environment for the growth of fungi, therefore recognition and early intervention is advisable because of the progressive nature of the fungal infections.

Majority of the patients with SFFI, had a raised fasting blood sugar (FBS) level from the standard value of <100 mg/dL and had high post prandial blood sugar (PPBS) level (standard value <140 mg/dL). Occurrence of SFFI was found to be statistically significant with less controlled FBS and PPBS levels respectively ( $P$  values were 0.015 and 0.003). Previous study of Delamaire *et al.*,<sup>[27]</sup> have shown that the elevated sugar levels in blood, decreases the granulocyte function, leading to tissue invasiveness and enhanced growth of superficial fungi in diabetic foot. Good metabolic management and maintenance of an optimally balanced concentration of blood glucose will help to delay and reduce the incidence of SFFI in lower limbs. Fungal re-infection was found in 73% indicating incomplete eradication of the fungal infection. Patients tend to stop treatment as soon as the swelling, itching and redness subside before the infection is fully eradicated resulting in possible re-infection. The data gathered from the questionnaire indicated gaps in knowledge with regard to the self-diagnosis of superficial mycosis which carry a

possible risk of inappropriate treatment. Similar, findings were documented in Maruyama *et al.*<sup>[28]</sup> These findings suggested the inadequate knowledge of the patients.

Also, may be due to less prominent symptoms associated especially with nail infections. Hence examining feet for fungal infections among diabetic patients is important because it might lead to development of severe secondary bacterial infection in diabetic foot. Therefore, education of diabetic patients regarding appropriate foot hygiene and the need of daily self-inspection of the feet in order to detect and manage the infections in lower limbs is important. Also, patients should be educated to prevent recurrence. Reporting any changes such as hard, brittle nails, discoloration of the nail and skin, whitish scales of the toe webs, to medical doctors at the diabetic clinic, cutting the toe nails short, drying the feet after a bath and avoid walking bare foot, using appropriate footwear and avoiding foot trauma should be encouraged in order to prevent SFFI in diabetes.

## CONCLUSION

*Aspergillus niger* was the predominant pathogen causing fungal foot infections in diabetic patients followed by *Candida albicans*. Fungal foot infections were seen significantly with the increasing age, gender, duration of the diabetes and with less controlled glycaemic level. Regular examination of the lower limbs and appropriate treatment is recommended in-order to minimize the possible complications associated with diabetic foot.

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