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Original Research Article

Diabetic foot ulcers and *Candida* co-infection: a single centered study

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Diabetic foot ulcers, *Candida* co-infection, *C. albicans*, Diabetes mellitus

A B S T R A C T

The aim of the study was to estimate the prevalence of *Candida* infection in Diabetic foot ulcer and also to describe the spectrum of *Candida* species. This study was carried out on diabetic patients with foot ulcer who visited our tertiary care hospital. A total of one hundred and forty two patients who satisfied the following criteria were included in this study. The age of the 142 patients with infected diabetic foot ulcer ranged from 38 to 67 years. There was no significant association of DFU occurrence with respect to age group and duration of diabetes [P>0.05]. Among the 142 samples obtained from diabetic patients with foot ulcer, 22 (15.49%) were positive for *Candida sp* by culture. Among the 22 *Candida* isolates obtained from 142 samples, *Candida albicans* was found to be the predominant species. Out of 22 *Candida* isolates, *C. albicans* was identified in 14 (63.63%), *C. tropicalis* in 4(18.18%) *C. parapsilosis* in 2 (9.09%). A single isolate of *C. guillermondii and C. krusei* was isolated from two different samples accounting for 4% and 5% each.

Introduction

Diabetes mellitus is the most common metabolic disorder and it is rising to pandemic proportions. Currently India leads the world as it has the largest diabetes population in the world. The estimate of 41 million people with diabetes in India is expected to rise to 66 million by 2025. As a consequence, the burden of diabetic foot disease is on the rise as well (Mohan *et al.*, 2007). The most devastating chronic complication of diabetes is diabetic foot. The alarming fact that peripheral neuropathy and peripheral vascular disease (PVD) which are present in >10% of the cases at the time of diagnosis in India can further increase the impact of diabetic foot (Pendsey *et al.*, 2010). The hallmark of Indian diabetic foot is severely infected foot. Such patients have life threatening infection and therefore invariably require primary limb amputation.

Eighty-five percent of these lower-limb amputations are preceded by polymicrobial infections of the wound. Increased vulnerability of these patients to infections can be attributed to impaired leukocyte function associated with vascular diseases, poor glucose control and altered host response (Bhatia *et al.*, 2003).

Bacteriology of DFU has been documented by various researchers and reported mixed aerobic - anaerobic microbes. However, literature references on the prevalence of fungal isolation from the diabetic foot ulcer are very limited. Candida spp. is the most commonly isolated yeast from these ulcers 5%-21% (Chincholikar and Pal, 2002; Bansal *et al.,* 2008; Dereume *et al.,* 1985). Although few studies have reported *Candida albicans* as the most prevalent species, others observed a clear shift towards non-albicans species. As these opportunistic fungi may invade deep into the wounds and delay healing of wounds. There have been some reports of an increased incidence of fungal infections (dermatophytosis and candidiasis) of inter-digital spaces and nails in the toes of diabetic patients, as well as of the association of these infections with the development of severe and deep inflammatory processes in feet (Gupta and Humke, 2000). White blood cells ability to destroy the pathogenic fungi due to fluctuating blood sugar and hypoxia from poor circulation increases the risk of acquiring infection in diabetic ulcer (Stadelmann *et al.,* 1998). The aim of the study was to estimate the prevalence of *Candida* infection in diabetic foot ulcer and also to describe the spectrum of *Candida* species.

Materials and Methods

Study population: This study was carried out on diabetic patients with foot ulcer who visited our tertiary care hospital from June 2010 to June 2012. A total of one hundred and forty two patients who satisfied the following criteria were included in this study. Age and duration of diabetes were recorded for all the patients after obtaining informed consent from the patient. Ethical committee clearance was obtained. Wagner's grading was recorded for classification of foot infections. Foot ulcers in diabetic patients were categorized into six grades (grade 0 – grade 5) based on Meggit Wagner Classification System (Bailey *et al.,* 1985). The criterion for inclusion was a diabetic patient with foot ulcer of grade 1 or more. However, all patients with grade 0 and/or limb amputations were excluded from the study group.

Specimen collection

Specimens like pus, wound exudates or tissue biopsy were obtained from the ulcer region. After thorough debridement, tissue specimens were taken from the depth of the wound. The samples were immediately transported to the lab.

Sample processing for fungal culture

The received samples were immediately observed after gram staining and KOH (10%) mounts. They were also inoculated into two slants of Sabouraud's Dextrose Agar, incubated at 30°C and 37°C and observed for 4 weeks and those showing growth resembling *Candida* species were further inoculated onto CHROM Agar. Urease test and germ tube test were also performed for identification of the yeast.

Data management and analysis

Data were entered into a database designed using MS Excel spreadsheet and analyzed using SPSS statistical software package [version 20]. Proportions for categorical variables were compared using chi-square test. P value less than 0.05 was considered statistically significant.

Results and Discussion

Among the 142 samples obtained from diabetic patients with foot ulcer, 22(15.49%)

were positive for *Candida sp* by culture **(**Table 1). The age of the 142 patients with infected diabetic foot ulcer ranged from 38 to 67 years. Most common age groups were 51–60 (56.33%), followed by >60 yrs, 49 (34.5%), 40–50 (7.74%) and < 40 (1.4%). Among the 86 (60.56%) male patients, highest number 43 (30.28%) of cases was found in 51–60 age group. Highest prevalence 37 (26.05%) of diabetic foot ulcer in females was found to be in the age group 51–60 yrs. Statistical analysis of prevalence of DFU with the age group did not reveal any significant association (Figure 1). Duration of diabetes data among the culture positive patients revealed, 63.63% had history for less than 10 years. More than 10 years of diabetes was found in 22.72%. There was no statistically significant difference in the prevalence of *Candida* infection in DFU with respect to duration of diabetes (P>0.05) (Table 2). Among the 22 *Candida* isolates obtained from 142 samples, *Candida albicans* was found to be the predominant species. Out of 22 *Candida* isolates, *C. albicans* was identified in 14 (63.63%), *C. tropicalis* in 4 (18.18%) *C. parapsilosis* in 2 (9.09%) samples. A single isolate of *C. guillermondii and C. krusei* was isolated from two different samples accounting for 4% and 5% each (Figure 2). The epidemic of diabetes mellitus is rising due to uncontrolled urbanization and changes in standard of living (Shubha *et al.,* 2013). It has increased from 2% in 1970 to 12% in 2000 in urban areas.

About 15% of diabetic patients during their lifetime develop foot ulcer and infection of foot ulcer is the most dangerous complication and is the frequent cause of hospitalization of diabetic patients. Polymicrobial infections of ulcer are responsible for lower limb amputation in 85% of the diabetic patients (Armstrong and Lipsky, 2004). Many studies have been conducted on the prevalence of bacterial infections of foot ulcer. However, literature references on fungal infections are very limited (Kates *et al.,* 1990). Therefore, little data is available on the magnitude of bioburden of *Candida* co infection in diabetic foot ulcer (Viswanathan *et al.,* 2002).

In the present study, we analysed the prevalence of fungal co-infection in diabetic foot ulcer. Out of 142 patients, samples from 39 patients had shown growth for *Candida sp.* accounting for the prevalence rate of 27.46%. This observation is compatible with the data reported by Gopi Chellan *et al.* (2012) and Saba Fata *et al.* (2011) showed the prevalence of 20.84% (108 *Candida* isolates out of 518 samples) and 19.9% (23 out of 120 samples). Our findings are higher than those found by Mallol *et al.* (1980) who reported the presence of *Candida* in 4.5% of 290 ulcers and 1.1% by Janifer *et al.* (2013).

In this study, the age group of 51–60 years showed the highest prevalence of diabetic foot ulcer (56.33%), followed by age group of >60 yrs (34.5%). However, statistical analysis of DFU prevalence with respect to age group did not reveal any significant association (p > 0.05). It has been observed that 60.56% were males and 39.43% were females similar to the findings by Piérard and Piérard-Franchimont (2005) diabetic foot ulcer were found to be more common in males compared to females. The supportive data was seen in other similar studies (Ahmed and El-Tahawy *et al.,* 2000; Sharma *et al.,* 2006; Hena and Growther *et al.,* 2010; Hayat *et al.,* 2011), as they indicate males dominate in having diabetes with foot infections when compared to females. This could be due to gender related factors that affect the skin and differences in professional activity predispose the male patients to foot ulcer.

Table.1 Prevalence of *Candida sp* in diabetic foot ulcer

S.no	Total no. of patients screened	*Candida sp* grown on culture	% prevalence of *Candida* co-infection
1.	142	22	15.49

Figure.1 Age and sex distribution of patients with diabetic foot ulcer

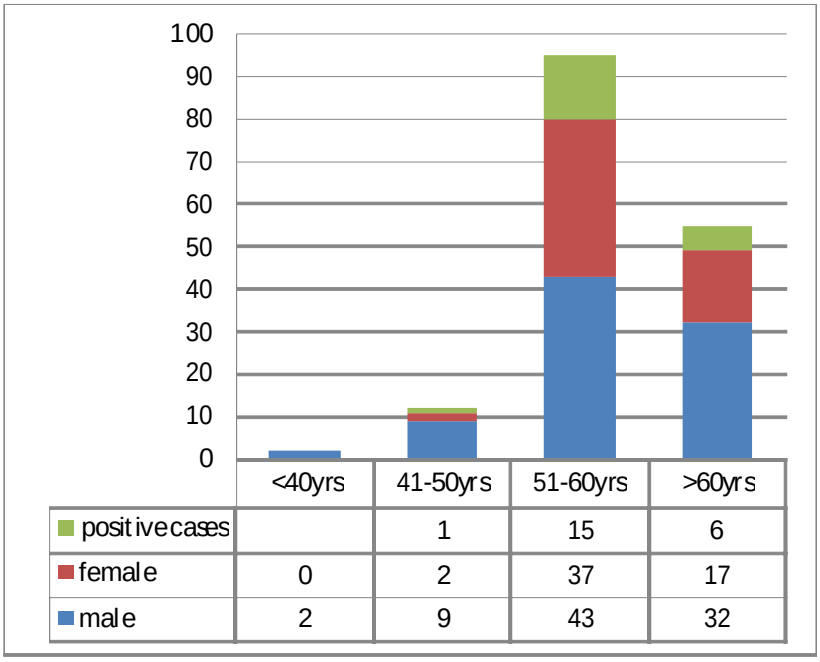
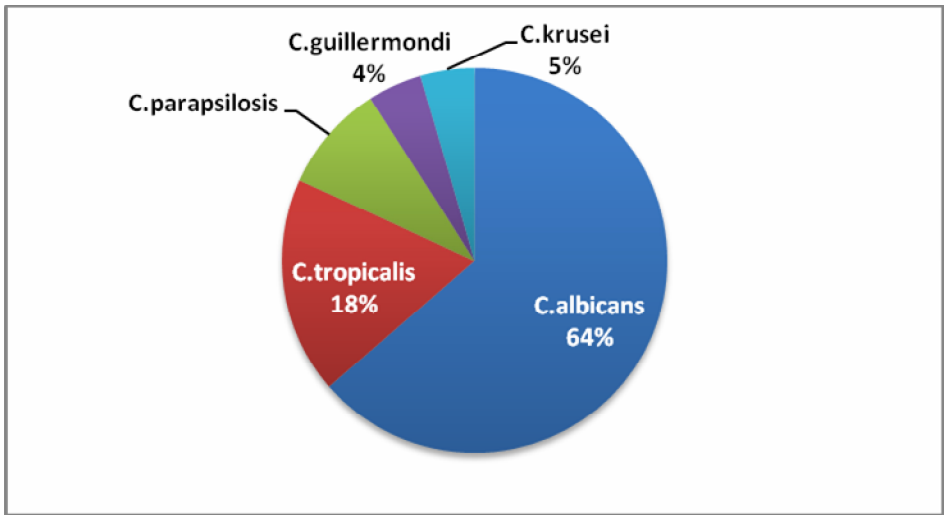


Table.2 Duration of diabetes mellitus at the time of presentation of foot ulcer

Duration	No. of patients	No. of culture positive cases	% of total
< 1	1	NIL	0
1-5	26	3	13.63
6-10	92	14	63.63
>10	23	5	22.72

Figure.2 Distribution of *Candida* species in diabetic foot ulcer [DFU]



Our results showed that *Candida albicans* were the most frequently isolated species among the 33 species accounting for 63.63% of total candidal infection, followed by *C. tropicalis* in 4 (18.18%) and *C. parapsilosis* in 2 (9.09%). A single isolate of *C. guillermondii and C. krusei* was isolated from two different samples accounting for 4% and 5% each.

It is worth mentioning here about *C. dubiliensis*, species of clinical importance as most the strains exhibit inherent azole resistance. Interestingly enough, we have not observed a single strain during the study period.

Nair *et al.* (2007) who showed 49% of *C. albicans*, 23% of *C. tropicalis* and 18% *C. parapsilosis*, reported a similar finding. Although the association of *Candida sp* with diabetic foot ulcer have been demonstrated by different similar studies (Chincholikar and Pal, 2002; Heald *et al.*, 2001) spectrum of fungal species differs in each, might be due to geographical and time based variation.

In contrast Bansal *et al.* (2008) from India in her study on 103 patients with diabetic foot wounds had reported *C. tropicalis* (29%) as predominant species which was followed by *C. albicans* (14%), and *C. guillermondii* (7%). The same spectrum of fungi was isolated from immune-compromised patients' blood by Pfaller *et al* and Bedeni *et al.*

Similarly, Missoni *et al.* (2005) from Croatia had also reported the fungal incidence in tissue biopsy specimens of 22 diabetic patients who had clinical evidence of fungal infections. The predominant isolates were *C. parapsilosis* (45.5%), *C. tropicalis* (22.7%), *C. albicans* (9.1%) and *C. glabrata* (9.1%). Studies have reported that *C. parapsilosis* has dramatically increased in significance and prevalence over past two decades and is known to be one of the leading causes for invasive *Candidal* disease.

This conflicting data can be explained by differences in exposure time to infection agents and differences in climatic and socio-economic factors in the respective geographical areas.

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