Incidence and Prevalence of Candidiasis in Diabetic Foot Ulcer Patients

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INTRODUCTION

Diabetic foot ulcers (DFUs) are widely in the community with prevalence rates ranging from 5% to 10%. Up to 25 percent of people with diabetes develop foot ulcer in their lifetime. Microorganism causing infections in DFU are bacteria, few studies reported the fungus and low pathogenic yeast (Singh et al., 2005; Berkow and Lockhart, 2017). The role for fungal infection in the pathogenesis of diabetic foot lesions has been suggested previously but remains unstudied. In diabetic foot, low pathogenic yeasts may cause an foot ulcers infection. These types of yeasts often belong to the normal mycobiota of the skin around ulcers, colonize diabetic foot ulcers secondarily, hindering the assessment of the real role of fungal isolates from the ulcer (Bansal et al., 2008). Some reports shows an increased incidence of fungal infections of interdigital spaces and nails in the toes of diabetic patients, association of these infections leads to the development of severe and deep inflammatory processes in feet (Richards et al., 2001). Candida a spp. is the common yeast isolated from diabetic foot ulcers with a prevalence of 5%-21% (Chincholikar and Pal, 2002; Bansal et al., 2008). Fluconazole used as the main option for the treatment of Candida albicans. Resistance to fluconazole are increasingly reported and it is a public health issue (Chellan et al., 2010; Nithyalakshmi et al., 2014). Diabetic foot syndromes are one of the main causes of morbidity (Mayfield et al., 1998). Opportunistic
fungal Infections was not given importance in the present scenario, compared to its bacterial portion because of lack of researcher. Though, topical studies show the broad range of fungal strains in diabetic infected foot ulcer patient, with Candida species is the commonly isolated strain. Treatment of an infected diabetes foot ulcer should encompass all the possible microbiological causes, to provide efficient and specific treatment to the patients (Ajello et al., 1998). Fluconazole is recommended as the primary main therapeutic option for the treatment of Candida albicans (Berkow and Lockhart, 2017). However, inherent and acquired resistances to fluconazole are increasingly being reported and are a serious concern. (Chellan et al., 2010). Protracted therapy and increased use for recurrent candidiasis are risk factors for the development of resistance to fluconazole (Bansal et al., 2008). Therefore, the main objective of our present study, find out the incidence and prevalence of candidiasis in diabetic foot ulcer patients.

MATERIALS AND METHODS

This was a prospective study done on diabetic patients with DFU who visited the outpatient (OPD) surgical department at tertiary care hospital Puducherry from January 2016 to June 2016. The study was carried out after the institutional human ethical committee clearance. Patients with diabetic foot ulcers visiting our OPD were included in this study. Patients treated with antifungal therapy, chemotherapy and corticosteroids were excluded. A total of 100 samples were collected from diabetic foot ulcer patients. Two tissue samples were collected from a deep ulcer, place the tissue in normal saline and sent to the laboratory for further processing. Microscopic examination of tissues was done. First tissue was placed in 10% KOH, second tissue used for fungal culture with Sabouraud’s dextrose agar (SDA) supplemented with Chloramphenicol and cycloheximide, incubated at 30°C for four weeks. Based on colony morphology, Gram stain was performed to rule out the bacterial isolates.

Identification of Candida species

Hi CHROM agar plates were incubated at 30°C for 24-48 hours. Species were identified based on the color of the colony.

Candida albicans — Light green
Candida glabrata — cream to white
Candida krusei — purple fuzzy and blue to purple
Candida tropicalis — Candida tropicalis.

Germ tube test

Take 0.5 ml of human serum in a test tube and inoculate 1-2 isolated colony, incubate at 37°C for 2 hours. Observe the germ tube formation under the microscope after 2 hours.

Cornmeal agar for Chlamydospore formation (Dalmau plate)

Divide the Cornmeal Agar plate into four parts. Using a needle, touch the isolated colony and then make 2-3 streaks. Place a cover glass to the control part. This will provide an anaerobic environment. Plates are incubated at 25°C for 2-5 days. Place the plate in a microscope and focus the edge of the cover glass under the 40X objective. Observe morphological features of Candida species.

Sugar Fermentation

Prepare sugar fermentation medium. Add 2% of sugar to the medium and place sterile Durham’s tube for gas production. Inoculate each sugar fermentation broth with 0.1 ml of inoculum. Incubate the tubes at 25°C up to 1 week. Examine the tubes every 48-72hrs period for the acid and gas production in Durham’s tube. Production of gas in the tube is taken as fermentation positive, and acid production indicates that carbohydrate is assimilated.

Assimilation Test

Suspend a heavy inoculum of a yeast culture that has been subculture on sugar-free medium in 2ml of Yeast Nitrogen Base. Place the carbohydrate impregnated discs onto the agar surface. Incubate the plates at 37°C for 3-4 days. The presence of growth around the disc is considered as positive.

Antifungal Susceptibility Test

The antifungal sensitivity testing of yeast isolates was carried out using the disk diffusion method as per CLSI guidelines. Mueller Hinton agar supplemented with 2% glucose, and 0.5μg/ml methylene blue was used for sensitivity testing. 3-4 isolated yeast isolates prepared inoculums. Inoculum suspension was adjusted to 0.5McFarland standard. Inoculate the Muller hinter agar with a suspension using a sterile cotton swab by lawn culture method. The plates were allowed to dry, and antifungal discs were placed onto the surface of the inoculated agar plate.

RESULTS AND DISCUSSION

Out of 100 DFU cases, Candida species were isolated in 32(32%) patients. It was more significant in males 22(68.75%) than females 10(31.25%) in Table 1. Age of the patients from 40 to 69 years was more infected. Out of 100 samples collected from patients with diabetic foot ulcer 32 (32%), isolates
Table 1: Gender distribution of Candidiasis in DFU cases.

| Total Sample | Positive Candida Cases in DFU | Male | Female |
|--------------|-------------------------------|------|--------|
| 100          | 32 (32%)                      | 22 (68.75%) | 10 (31.25%) |

Table 2: Prevalence of Candida sp in diabetic foot ulcer.

| S No | Total Positive Cases (n=100) | % prevalence of candida species |
|------|-----------------------------|--------------------------------|
| 1    | 32                          | 32%                            |

Table 3: Distribution of candida species in diabetic foot ulcer.

| Candida species | No of culture positive cases (n=32) | Percentage (%) |
|-----------------|-------------------------------------|-----------------|
| C.albicans      | 16                                  | 50%             |
| C.tropicalis    | 8                                   | 25%             |
| C.parapsilosis  | 5                                   | 16%             |
| C.krusei        | 3                                   | 9%              |

Table 4: Antifungal Resistant Pattern (%) of Candida species.

| Anti Fungal Drug | Candida albicans (n=16) | Candida tropicalis (n=8) | Candida parapsilosis (n=5) | Candida krusei (n=3) |
|------------------|-------------------------|--------------------------|----------------------------|---------------------|
| Fluconazole      | 10 (62.5%)              | 4 (50%)                  | 2 (40%)                    | 1 (33%)             |
| Amphotericin B   | 8 (50%)                 | 2 (25%)                  | 2 (40%)                    | 0 (0%)              |
| Voriconazole     | 6 (37.5%)               | 2 (25%)                  | 1 (20%)                    | 0 (0%)              |
| Itraconazole     | 4 (25%)                 | 0 (0%)                   | 0 (0%)                     | 0 (0%)              |

of Candida were isolated shown in Table 2. Among the 32 Candida isolates obtained from 100 samples, Candida albicans was found to be the prime species. Out of 32 Candida isolates, 16 (50%) C.albicans, 8 (25%) C.tropicalis, 5 (16%) C.parapsilosis and 3 (9%) C.krusei were isolated in DFU cases Table 3. Out of 16 C.albicans isolates, 10 (63%) was resistant to fluconazole and 16 Non-albicans sp isolated, 7 (44%) were resistant to fluconazole shown in Table 4. About 17% of diabetic patients develop a foot ulcer in their lifetime. It is one of the primary cause of hospitalization for diabetic patients. 85% of Poly microbial infections of ulcer are responsible for limb amputation in diabetic patients (Armstrong and Lipsky, 2004).

Several studies have been conducted on the bacterial infections of foot ulcer. Literature references for fungal infections are minimal still (Kates et al., 1990). Thus, little data is available on Candida Co-infection in diabetic foot ulcer Viswanathan et al. (2002). In our present study, among 100 DFU cases, 32 (32%) Candida species were isolated. It was more significant in males 22(68.75%) than females 10(31.25%). Diabetic foot ulcer patients age range from 40 to 69 years. It has been observed that 57.5% were males, and 42.5% were females which is similar to the result (Pierard and Pierard-Franchimont, 2005). The accommodating results were shown in other similar studies by (Hena and Growther, 2010) males leads in having diabetes with foot infections when compared to females.

In the present study, we analyzed the fungal co-infection in foot ulcer. Among the 32 Candida isolates from 100 samples, Candida albicans was found to be the prime species. 16 (50%) C.albicans, 8 (25%) C.tropicalis, 5 (16%) C.parapsilosis and 3 (9%) C.krusei isolated in DFU cases. Our results are higher than those reported (Jasmine et al., 2013). Out of 16 C.albicans isolates, 10 (63%) was resistant to fluconazole and 16 Non-albicans sp isolated, 7 (44%) were resistant to fluconazole. Antifungal drugs resistant to Candida sp with diabetic foot ulcer relates (Richards et al., 2001).
late with (Martinez et al., 2002). Resistance to antifungal agents was comparable to previous studies with amphotericin resistance 7%, fluconosine 7.9%, and voriconazole 4%. It is unclear at present, due to limited use of these agents in the community compared to fluconazole.

CONCLUSIONS

Our results show a Candida species resistant to fluconazole in DFU is a significant concern due to the inappropriate use of drugs in diabetes patients. Due to lack of Oral antifungal agents for treating fungal infections, which makes it important to prevent the spread of resistance. Increase in resistance is a significant public health concern for the use of fluconazole in the community. Our results will make physicians easier to treat fungal and mixed infections of diabetic foot ulcers and encourage further research into these infections.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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