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Detection of *Candida* spp.in Diabetic Septic Foot in Khartoum State Diabetic Center

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Abstract

Introduction: Diabetes mellitus is a chronic disorder affects immune system beside other systems and causing premature death or complications within time causing patients suffering. Because of immune system involvement, fungal infection is one of the prominent infections especially if patients presented with wound.

Material and method: 50 DM patients, 20 females and 30 males were enrolled. Double wound swabs were collected from each patient. 1st one for direct microscopy (smear stained with 70% methylene blue). The 2nd one was used for culture in 3 test tubes containing Sabouraud Dextrose Agar, 2 test tubes were incubated aerobically (the 2 tubes used for confirmation).

Result: *Candida* spp. found in 9 (18%) patients. *Candida* spp. found among elder patients.

Conclusion: In this study we were able to isolate *Candida* spp. from diabetic foot ulcer patients.

Key word: Diabetes Mellitus, Candida.

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Introduction

In Sudan, diabetes is now common and major health problem. In North Sudan, in urban areas the evaluated prevalence of diabetes was thought to be around 19% in comparison with 2.5% in rural regions (1, 2). Recently, in Africa the prevalence of diabetes is predictable to increase up to 28 million cases by 2030 in comparison with 14 million in 2011 (3,4). According to the WHO and the IDF expected that the number of adults with diabetes in the world will rise near 629 million by the year 2045 (5, 6).

Diabetes mellitus (DM) is a long-lasting, non-communicable condition that raises blood sugar levels because of insufficient or ineffective insulin secretion (7). In addition, it is quickly emerging as one of the biggest modern threats to public health. (8, 9), Up to 15% of diabetic patients worldwide currently experience diabetic foot ulcers (DFUs), which are still very common among those with the disease (10, 11). The majority of microorganism-causing infections in DFU are bacteria, then few of filamentous fungi and low pathogenic yeast. (12, 13). While, *Candida* spp. is the most commonly isolated yeast from these ulcers with a percentage of 5%–21%. (14). It has previously been studied that the relationship between diabetes and candidiasis (15). due to the increased susceptibility

of diabetic patients to fungal infections compared to those non diabetic patients (16, 17). Recent evidence suggests that diabetic persons are more susceptible to periodontal disease , tuberculosis (TB), lung infection by *Legionella pneumoniae*, 'mucormycosis' caused by the *Mucoraceae* family of fungi (18, 19).

It has been described that *Candida spp.* compared to healthy subjects, is more common in patients with type 1 and type 2 DM with poor glycemic control. *Candida* species are the most frequent cause of fungal infections, which can result in a variety of fatal diseases (20).

(Raiesi.,*et al*) (21) in their study reported that (24.5%) of diabetic patients were affected with fungal infections. In addition, the prevalence of fungus infection was 19.1% in patients with diabetic foot ulcer and 28% of patients with skin and nail lesions. *Candida albicans* and *Aspergillus flavus* were the most common species isolated from thirty patients with fungal infection.

The aim of this study is to detect and estimate fungal infection among diabetic septic foot ulcer in diabetic centers in Khartoum state.

Materials and methods

Sample area and size: The study only performed among diabetic patients with foot ulcers. The study was carried out at Khartoum State diabetic care centers (Zinam hospital and Alarbaein hospital). 50 diabetic patients with foot ulcers, including 30 male and 20 female.

Collection of samples: Under hygienic conditions two sets of swab samples were collected from each patient, the 1st one for direct microscopy (smear stained with 0.1% methylene blue).

The 2nd one was used for culture in 3 test tubes containing Sabouraud Dextrose Agar, 2 test tubes were incubated aerobically (the 2 tubes used for confirmation).

The 3rd tube was incubated at 26^0C at room temperature.

Ethical clearance: Ethical approval of this study was taken by Research committee of Omdurman Islamic University.

Methodology

Wet preparation: Specimen was mix well, one drop (10) microliters of the specimen were removed by sterile pipette. One drop (10 µL) of the specimen were placed on a clean microscope slide with the patient's identification number. One drop (10 µl) of 10% Potassium hydroxide (KOH) were added directly to the drop of specimen on the slide. Coverslip was placed on the drops on the slide. Slide was placed on a bright field microscope, low power (10X) focus was used, and high dry power (40X) was scanned. Budding yeast or yeast with pseudohyphae was examine.

Culture preparation:

Sabouraud Dextrose Agar: Suspend 65 g of powder in1L of distilled water, sterilization in autoclave at 121◦c for 15 minutes, then cooled and 1ml chloramphenicol antibiotic (The antibiotic solution is prepared by dissolving 1g of powder in 5ml DW) was added.

Corn Meal Agar: Suspended 17.0 g of powder in 1L distilled water. Sterilization in autoclave at 121◦ C for 15 minutes

Cultivation of samples: Each samples were cultivated on Sabouraud dextrose agar and Corn meal agar. The culture tubes were incubated at 37°C for 48 h under aerobic conditions. Culture tubes with no growth were further incubated for 7 days before declaring them negative for Candida growth. Samples were stained to check out if they were alive or not, but they were dead as the pandemic of corona virus arrested all targets.

Isolation of fungi: Isolation was conducted by comparing morphology of the grown fungal colonies based on size, color, margins, elevation and spreading. The isolated fungi were first identified morphologically using a compound microscope.

Gram staining: Gram stain was done according to method described by Crickmore. *et al*. **(22)** Organisms colored with red were classified as Gram-negative organisms and violet colored were classified as Gram-positive organisms.

Statistical analysis plan: The numerical datawere collected and analyzed by means of statistical package of social science program (**SPSS**) version 26. The data analysis summarized the results in statistical form, providing the means and the ranges of the data collected.

Results: In this study, 50 diabetic patients with foot ulcers, 30 were male and 20 were female, 9 of the patients which was 18% of the study population had fungal infections which revealed to *Candida* .spp. and the rest were 41(82%) with no *Candida* .spp. as shown in Table 1 and Fig 1.

Microscopic examination revealed Gram positive yeast, round and oval yeast, some of them showed budding with purple color appearance. *Candida*. spp. showed oval and consisted of mother cells, daughter cells by using methylene blue. Cultural characteristic of *Candida* spp. on Sabouraud dextrose agar whitish to creamy color and smooth soft colonies, and on Corn meal agar it was clear two forms of chlamydo-spores and blasto-conidia.

Table 1: Types of fungal infections among DM patients

Type of fungal infection	Number	%
Infection with *Candida* spp.	9	18%
No infection with *Candida* spp.	41	82%
Total	50	100%

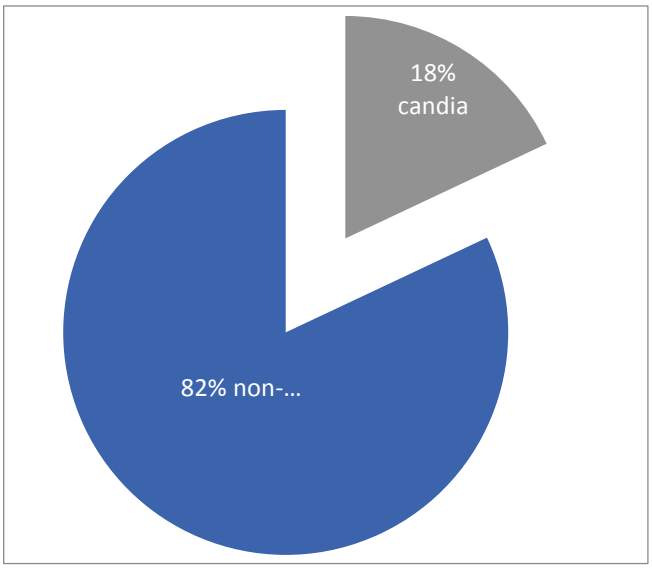

Figure 1: Types of fungal infections among DM patients

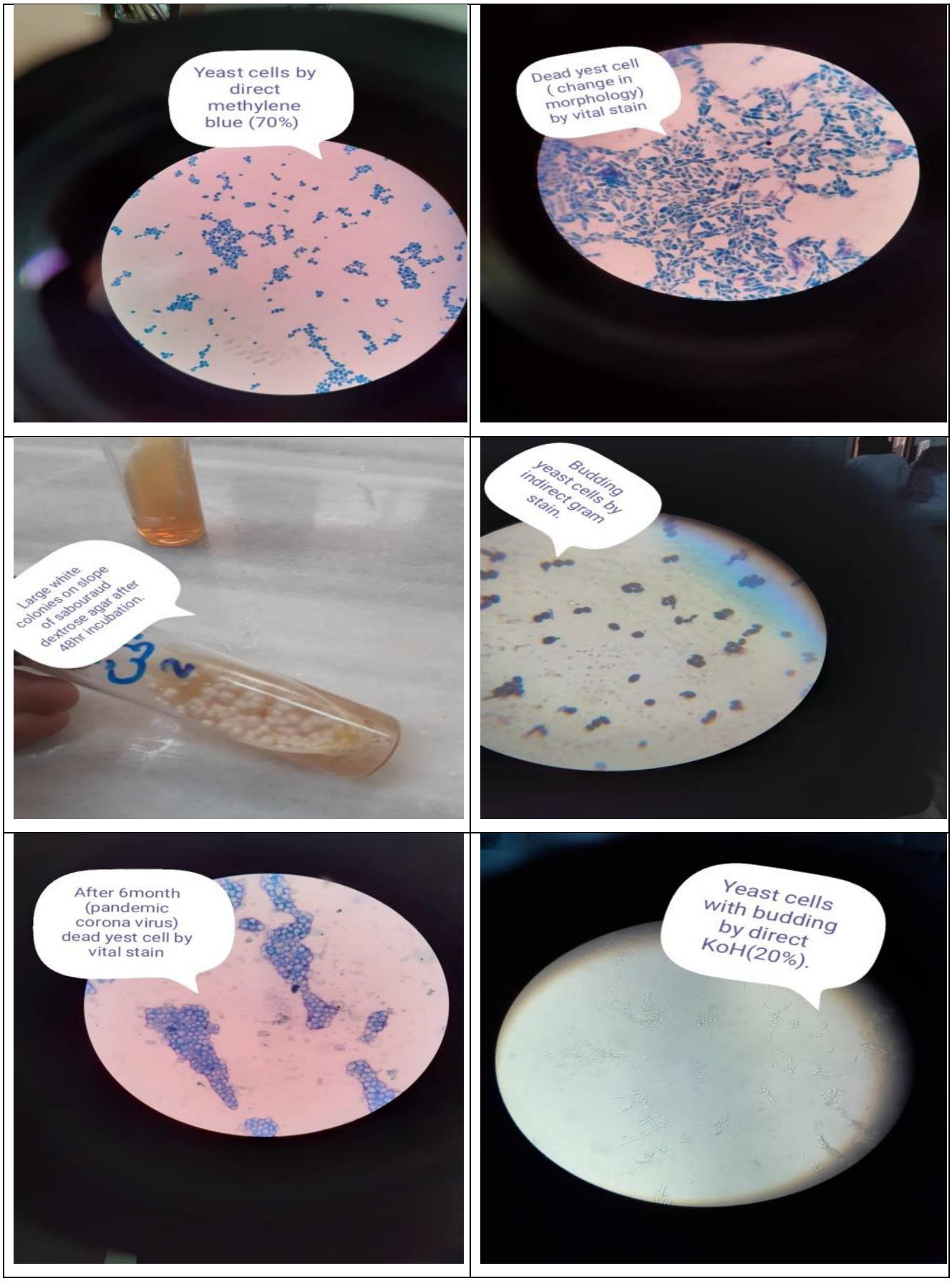

Figure 2: Wet preparation, Gram stain and Culture of samples

According to age, patients were classified into three group's first group from 29-50 years old 2 of them had shown *Candida* spp. infection. Second group 51-70 years old 4 of them shown *Candida* spp. Infection and the last group 71-90 years old 3 of them were recorded as *Candida*. spp. infection As shown in Fig 3.

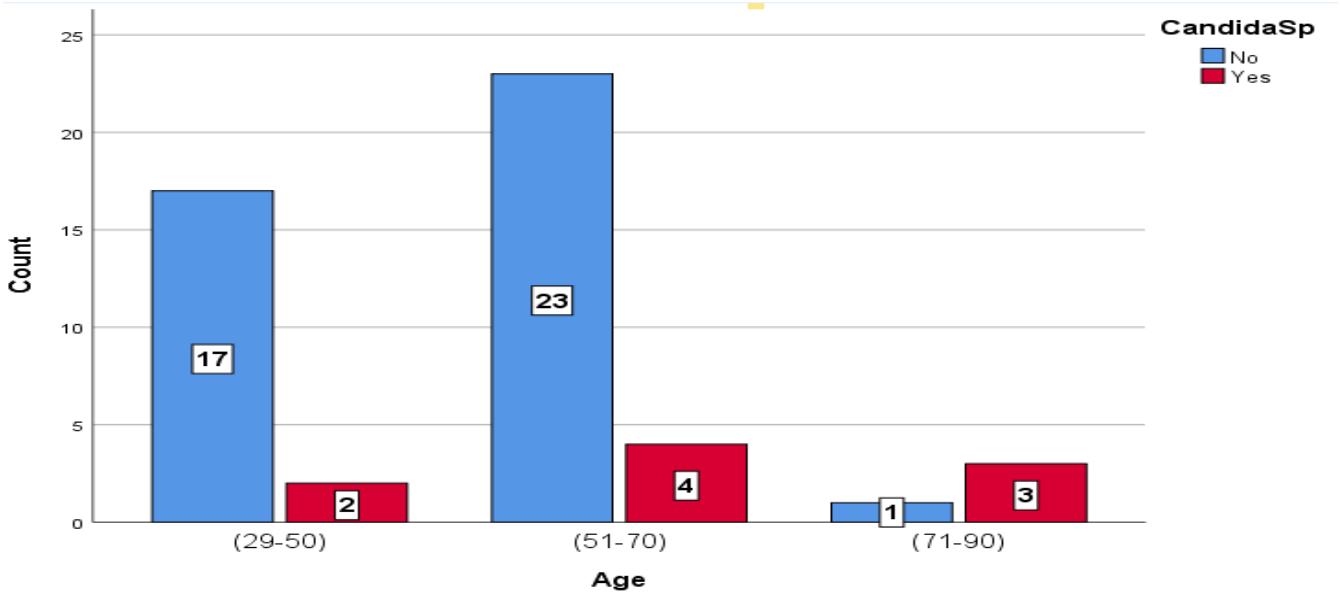

Figure 3: Percentage of infection with *candida spp* among age groups

Considering gender, 23 males presented with non-candida infection, 7 with *Candida*. spp, females 18 with non-*Candida* infection and 2 with *Candida*. Spp. as in Figure 4.

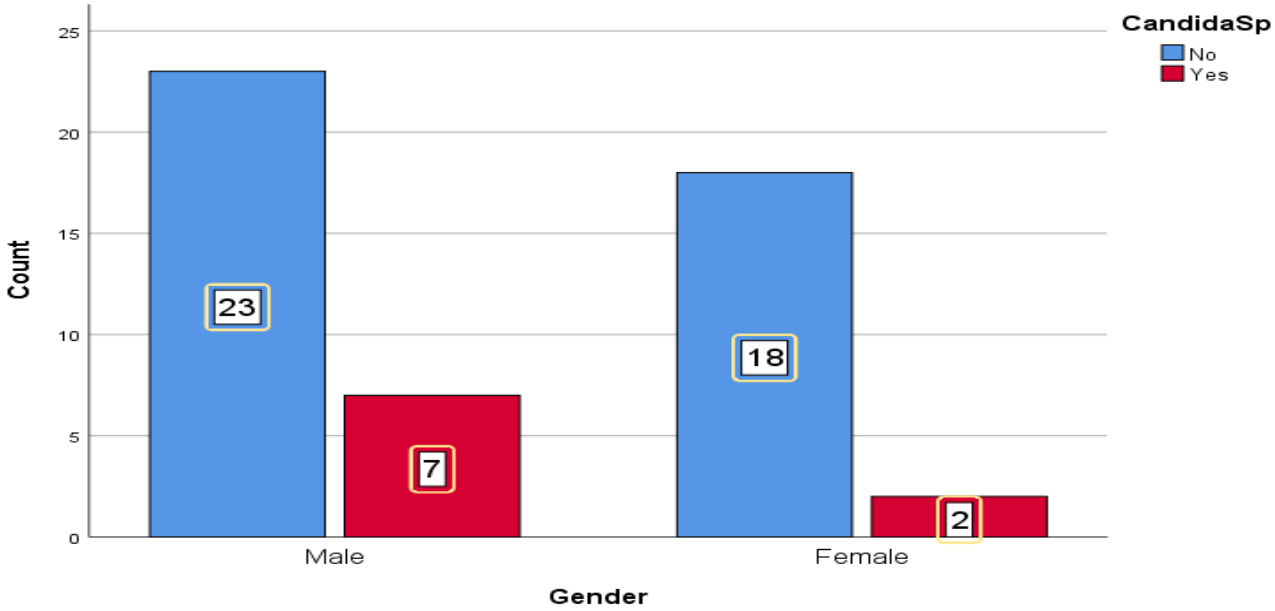

Figure 4: Gender distribution of fungal infection among DM patients

Pearson's correlation with age of diabetic patient's duration of wound infection among diabetic patients, it was negative correlation as in Figure 5

R value -.15

Figure 5: Correlation between age and duration of disease among DM patients

Discussion

We believe that microbiological profile of diabetic foot ulcer patients ranges from chronic bacterial infections to opportunistic fungal infections. Immuno-compromised patients are known to be susceptible for fungal infection and are a major public health concern worldwide

However, fungal infections in diabetic foot ulcers infections have not been studied extensively.

(DFU) diabetic foot ulcer, it is a chronic disease that affect skin and tissue damage in a diabetic patient, which is difficult to heal.

The fungi involved in diabetic foot ulcers are mainly *Candida* spp. Other species commonly isolated are *Trichophyton* spp. and *Epidermophyton Floccosum*.

It was clear that diabetic patients with fungal foot infection were correlated with age, sex and duration of diabetes Eckhard *et al*. (23). In the present study, the majority of patients were males 60% which is similar to that observed in other studies Eckhard., *et al* (23). and Sanniyasi., *et al* (24). Considering age and duration of wound infection, a negative correlation presented as R value was -0.15. The result of this study, indicated fungal infection was (18%) which is higher compared to Sanniyasi., *et al* (25) who recorded 16.2% in diabetic patients, but these percentages are higher than other study conducted by Mehra .,*et al* (26) with (11.43%) . In addition, a previous studies done by both Fata *et al* (27)and Bansal *et al*(14) Reported (52%) and (50%) respectively positivity *Candida* spp. in their cultures. Recently, very high prevalence of *Candida* spp. recorded by Abilash *et al* (28)(88.8%) in diabetic foot ulcer.

Unfortunately our further investigation to determine the Candida species not completed due to Corona virus shutdown.

In conclusion, the results of this study demonstrated that the majority of fungal infections that isolated from diabetic foot ulcer patients were *Candida*. spp.

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