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Incidence of Mycotic Infections in Diabetic Patients Attending Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria.Shinkafi, S.A.¹, Mohammed, N.¹, Abba, O.¹, Hauwa'u B.A.² and Bello, L.³Department of Biological Sciences, Federal University Gusau, Zamfara State Nigeria¹,Department of Microbiology Sokoto State University, Sokoto Nigeria²,Department of Microbiology Usmanu Danfodiyo University Sokoto, Nigeria³.

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Abstract

The study was carried out to investigate the incidence of mycotic infections in diabetic patient. A total number of twenty-five (25) diabetic patients with malignant diabetic infections were examined. Among the 25 patients sampled, (sixteen) or 64% patients were found positive for one fungus or the other. Germ tube tests, test for potassium hydroxide and urease test were carried for isolation of fungi. A total number of four fungal species were isolated. These include; *Candida albican*, *Candida tropicalis*, *Candida parapsilosis* and *Trichosporon mentagrophyte*. *C. albicans* had the highest percentage frequency of occurrence of 32% followed by *Candida tropicalis* with 16% and *Candida parapsilosis* had 12%, while *Trichosporon mentagrophyte* had the least percentage of occurrence of 4%. The result of patients with diabetic foot ulcers in relation to different age groups revealed age group sixty years and above to have the highest percentage frequency of 44%, the age group less than 40 had the least percentage prevalence of 8%. Based on the results obtained in this research it was observed that age group sixty years and above had the highest percentage frequency of infection. This could be attributed to the low of weak immune system of the patients under this group. Based on the findings of the research it is recommended that more research on evaluation and treatment of chronic pathogenic biofilms are encouraged.

Keywords: Mycotic Infections, Diabetic Patients, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Introduction

Diabetes is a chronic metabolic disorder of the endocrine system which plaques a large segment of people and constituting a major public health problem worldwide. Diabetes and foot problems are almost synonymous (Fryberg *et al.*, 2000). World Health Organization (WHO) report indicates that India has the largest diabetic population (19 million in 1995) that is expected to rise to 57 million by 2015 (Mayfield *et al.*, 1998). A commonly accepted definition of foot infection is the presence of systemic sign of infection e.g., fever and leucocytosis, purulent secretions or, two or more local symptoms or signs such as redness, warmth, indurations, pains or tenderness (Kahn *et al.*, 2007). Diabetic foot is one of the most feared complications of diabetes and it is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infections with or without osteomyelitis, leading to development of gangrene and even necessitating limb amputation (Alavi *et al.*, 2007). Fifteen percent of people with diabetes will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer (Nwachukwu *et al.*, 2009).

A mycotic infection is a disease caused by pathogenic fungi. Fungi are a group of non-motile eukaryotic organisms which exist as saprophytes, parasites or commensals. They can either cause superficial, subcutaneous, systemic, opportunistic or miscellaneous mycoses (Arora *et al.*, 2008). Infection with fungi is often a consequence of predisposing factors including age, stress or other pathologic conditions (e.g.

cancer, diabetes, AIDS). Fungal infection among diabetic patients is one of the major health concerns worldwide (van Houtum *et al.*, 2008), but the spectrum of fungi infecting diabetic lower-limb wounds and their pathogenicity have not yet been studied thoroughly. Therefore, clinicians and surgeons treating diabetic foot wounds suspect only bacterial infections and treat them with antibacterial agents. They do not routinely send deep tissue from the wound bed for fungal culture and sensitivity, either due to lack of literature support or due to the assumption that there would not be any fungal infection in the diabetic wound. Opportunistic fungi may invade deep into the wounds and contribute to delayed wound healing in some of the diabetic patients who are otherwise immunocompromised compared to non-diabetics. Diabetics ulcers have 15 to 46 times higher risk of limb amputation than foot ulcers due to other causes (Khanolkar *et al.*, 2008). Every year more than a million diabetic patients require limb amputation (Alavis *et al.*, 2007). The impaired micro-vascular circulation in patients with diabetic foot limits the access of phagocytes favoring development of infection (Anandi *et al.*, 2004).

Viswanathan *et al.* (2000) reported 25% of diabetic individuals are anticipated to develop severe foot problems at some point in their life time that often ends with amputation. Diabetic foot infections are more severe and more difficult to treat than infections in non-diabetics. Polymicrobial etiology has been implicated in the infected diabetic foot.

The microbial contamination of diabetes wounds is an important cause of mortality (Mehta *et al.*, 2007). Though, the development of wound infection depends on the integrity and protective function of the skin, the wide spread used of antibiotics, together with the length of time over which they have been available have led to major problems of resistant pathogens in diabetic wound infections contributing to morbidity, and mortality (Nwachukwu *et al.*, 2009). It has been shown that wound infection is universal and the microbial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and examination

(Anupurba *et al.*, 2007). Infection is an important cause of mortality in diabetic wounds. It has been estimated that 75% of all deaths following injuries are related to infections (Mehta *et al.*, 2007). In general, wound can be considered infected if purulent materials drain from it, even without confirmation of positive cultures. Also, many wounds are colonized by fungi (including bacteria), whether infected or not. Infected diabetic wounds may not yield fungal pathogens by culture owing to the fastidious nature of some fungal pathogens, or if the patient has received an antifungal (Nwachukwu *et al.*, 2009). Many microbial agents (including fungi), are known to cause diabetic wound infections. Initial injury to the skin triggers coagulation and an acute inflammatory response (Yah *et al.*, 2010) followed by exposure of subcutaneous tissue following loss of skin integrity which provides a moist, warm, and nutritive environment that is conducive to microbial colonization and proliferation (Yah *et al.*, 2010; Shittu *et al.*, 2002). Opportunistic fungi may invade deep into diabetic wounds and contribute to delayed wound healing in some diabetic patients. Fungal infection among diabetic patients is one of the major health concerns worldwide (vanHoutum *et al.*, 2008), but the spectrum of fungi infecting diabetic lower-limb wound and their pathogenicity have not yet been studied thoroughly and, this often results in clinicians and surgeons concentrating their care of diabetic foot wounds only on bacterial infections the antibacterial agents only. They do not routinely send deep tissue from the wound bed for fungal culture and sensitivity, either due to lack of literature support or due to the assumption that there would not be any fungal infection in the diabetic wound. Diabetic foot is a result of uncontrolled diabetes and imperfect sanitary care which leads to necrotic lesions, gangrene and finally amputation. Secondary mycotic infections play a principal role to produce chronic lumpy lesions. This research is therefore, designed to investigate the incidence of fungal pathogens in diabetic foot infections, so as to be able to investigate know whether among the fungal pathogens there are opportunistic pathogens and recommend accordingly.

Materials and Method

Samples collection

The samples were collected with the aid of commercially prepared sterile swab sticks. Pus from diabetic wounds were aseptically collected and labeled. The samples were taken to the laboratory for analysis. The specimen was suspended in nutrient broth.

Preparation of culture media

The media used for this research was nutrient broth and Sabouraud Dextrose Agar both the media were prepared according manufacturers instruction

Nutrient broth (Fluka Biochemika 70122)

The media was prepared according to the manufacturer's instruction. Twenty- five grams of the powder was suspended in 1litre of distilled water. The medium was dissolved through heating. Ten milliliters were suspended into each test tube, cocked with cotton wool and then covered with aluminum foil. The test tubes were then sterilized by autoclaving at 121°C for 15minutes.

Sabouraud dextrose agar (SDA) (Titan Biotech Ltd)

The media was prepared according to the manufacturer's instruction. Sixty-five grams of the agar powder together with one gram of streptomycin were suspended in 1litre of distilled water. The medium was dissolved completely through boiling and was sterilized by autoclaving at 121°C for 15minutes. It was cooled to 45°C before dispensing into Petri dishes.

Sample processing

Samples collected from diabetic wound were inoculated onto Sabouraud Dextrose Agar (SDA). The plates were incubated both at room temperature (22-28°C) for up to four weeks and evaluated daily for growth of fungal cultures. Necrotic areas of the tissues were also mounted on Potassium Hydroxide (10% KOH) and analyzed by direct microscopic mycological examination.

Isolation and Identification of fungi

Yeast-like growths on SDA were evaluated

macroscopically, microscopically, germ tube formation, urease production and sugar assimilation test.

Germ tube test

A small portion of an isolated colony of the yeast to be tested was suspended in a test tube containing 0.5 ml human plasma. The test tube was inoculated and incubated at 35°C for not longer than 2 hours. A drop of the yeast-serum suspension was placed on a microscope slide, a cover slip was placed, and examined microscopically. Presence of germ tubes indicated a positive test (Alavi *et al.*, 2007).

Test for potassium hydroxide (KOH)

Samples from infected tissues were added separately onto clean glass slides. To each glass slide, a drop of 10%KOH was added and cover slip was gently placed. The slides were gently heated to speed up the action of the KOH. The slide was examined microscopically using x10 and x40 objective lens (Anandi *et al.*, 2004).

Test for Urease

Heavy inoculum of a pure fungal culture was streaked onto the entire surface of Christensen's urease slant. The tubes were incubated with loosened caps at 35°C. The slants were observed for color change at interval of 6 hours every day for up to 6 days. Urease production was indicated by a bright pink (fuchsia) color on the slant that extends into the butt.

Results

A total number of twenty-five (25) diabetic wounds samples were collected from the feet of different diabetic patients attending Usmanu Danfodiyo University Teaching Hospital, Sokoto (UDUTH). Among the 25 patients sampled, eighteen (18) were males and seven were females. Out of the 25 samples studied, 16 were positive for fungal growth while 9 yielded no growth. Table 1 indicated the results of biochemical tests conducted. A total number of four (4) fungal species were isolated. *Candida* species were the major isolated species. These include *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, and a dermatophyte *Trichosporon mentagrophytes*. Table 2 indicated the percentage frequency of occurrence of the

isolated organisms. *C. albican* had the highest percentage frequency of occurrence of 32%, followed by *C. tropicalis* with 16%, then *C. parapsilosis* with percentage frequency of occurrence of 12%. *T. mentagrophyte* had the least frequency of occurrence of 4%. The percentage of the results of patients with diabetic

foot ulcers in relation to different age groups are indicated in Table 3. Age group above sixty (60) years was found to have the highest percentage of occurrence of diabetic foot ulcers. 44%. The age group less than 40 years has the least percentage prevalence (8%) of diabetic foot ulcers.

Table 1: Biochemical characteristics of isolated organisms

Yeast species	Germ tube	Urease	Sugar assimilation					Microscopy
			Glu	Suc	Lac	Tre	Raf	
<i>Candida tropicalis</i>	-	-	+	+	-	+	-	No arthroconodia. Blastoconidia produced randomly along hyphae or pseudohyphae. Hyphae or pseudohyphae Branched.
<i>Candida albicans</i>	+	-	+	+	-	+	-	No arthrocononidia. Spherical clusters of blastoconidia at regular intervals on pseudohyphae. Chlamydo spores present on hyphae.
<i>Candida parapsilosis</i>	-	-	+	+	-	+	-	No arthroconidia. Blastoconidia present but not characteristic. Giant hyphae with sage brush appearance.
<i>Trichosporon mentagrophyte</i>	-	-	+	+	+	+	+	Numerous arthroconidia. Blastoconidia present, septate hyphae present.

Key: glucose (*Glu*), sucrose (*Suc*), lactose (*Lac*), trehalose (*Tre*), raffinose (*Raf*).

Table 2: Percentage frequency of occurrence of patients with diabetic foot ulcers in relation to different age groups

Age Groups (Years)	Male (%)	Female (%)	Total (%)
< 40	1(4)	1(4)	2(8)
41-50	3(12)	1(4)	4(16)
51-60	6(24)	2(8)	8(32)
>60	8(32)	3(12)	11(44)
Total	18(72)	7(28)	25(100)

Table 1: Percentage frequency of occurrence of fungi Isolated from patients diabetic foot ulcers

Fungal isolates	Number (%) frequency of occurrence
<i>Candida albican</i>	8(32%)
<i>Candida tropicalis</i>	4(16%)
<i>Candida parapsilosis</i>	3(12%)
<i>Trichosporon mentagrophyte</i>	1(4%)

Discussion

The research evaluated the prevalence of fungal infections in diabetic foot ulcers in patients attending Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Out of twenty-five (25) diabetic patients with diabetic foot infections studied, sixteen (16) were males and nine (9) were females. Predominance of male over female patients as shown in the study can be explained based on the difference in life style, propensity to micro traumatism, professional activities, sport practices, as well as cultural and religious beliefs which limits females' exposure to the external environment. The age group with the highest fungal isolate is 60 years and above accounting for a total of 11(44%) isolates (8 males and 3 females), this was followed by age group 51-60 with percentage occurrence of 8(32%). Age group with least recovery is 0-40 years. The reasons for high prevalence among the age group sixty (60) and above might be attributed to the fact that at older age, the competence of the immune system tends to be lowered thereby allowing fungal and other microbial proliferations. The results of this research shown in (Table 1) indicated that *Candida* spp. were the most frequent isolates which include *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *T. mentagrophyte*. Our finding is consistent with a previous report (Nair *et al.*, 2007) which indicated a higher prevalence of *C. albicans* and *C. tropicalis*. Our finding is however at variance with previous reports (Bansel *et al.* 2008; Chincholikar *et al.*, 2002; Heald *et al.*, 2001) which indicated that *C. tropicalis* as the predominant isolate. In diabetic patients, the presence of other species of *Candida*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei*, *C. tropicalis*, *C. famata*, *C. kefyr* and *C. glabrata* have been reported in diabetic patients by other investigators (Bansel *et al.*, 2008; Eckhard *et al.*, 2007; Hohaus *et al.*, 2003; Chincholikar *et al.*, 2002; Heald *et al.*, 2001). In contrast to this study, previous reports (Eckhard *et al.*, 2007; Romano *et al.*, 2001) demonstrated that dermatophytic infection in diabetic foot patients was more common than *Candida* spp. and reported *T. rubrum* as the most isolated fungi in these patients. 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. Furthermore, mold species *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Scopulariopsis* spp. were reported by other authors (Eckhard *et al.*, 2007, Nair *et al.*, 2007 and Tajaldeen *et al.*, 2006) as causative agents of fungal infections in the diabetic foot patients.

Conclusion

Based on the research conducted on the incidence of mycotic infections in diabetic patient. A total number of twenty-five (25) diabetic patients with malignant diabetic infections were examined. Among the 25 patients sampled, (sixteen) or 64% patients were found positive for one fungus or the other. Germ tube tests, test for potassium hydroxide and urease test were carried for isolation of fungi. A total number of four fungal species were isolated. These include; *Candida albican*, *Candida tropicalis*, *Candida parapsilosis* and *Trichosporon mentagrophyte*. *C. albicans* had the highest percentage frequency of occurrence of 32% followed by *Candida tropicalis* with 16% and *Candida parapsilosis* had 12%, while *Trichosporon mentagrophyte* had the least percentage of occurrence of 4%. The result of patients with diabetic ulcers in relation to different age groups revealed age group sixty years and above to have the highest percentage frequency of 44%, the age group less than 40 had the least percentage prevalence of 8%.

References

- Akinkugbe, O.O. and Akinyaju, O.O. (2005). Final report-National survey on non-communicable diseases in Nigeria. Federal Ministry of Health Lagos: 65-68.
- Alavi, SM., Khosravi A.D, Sarami, A., Dashtebzorg, A., and Montazeri E.A. (2007) Bacteriologic study of diabetic foot ulcer. *Pakistan Medical Science*; **23**:681-684.
- Anandi, C., Alaguraja, D., Natarajan, V., Ramanathan, M., Subramaniam, C.S. and Thulasiram, M. (2004). Bacteriology of diabetic foot lesions. *Indian Journal of Medical Microbiology*; **22**:175-178.
- Anupurba, S. Ahattachajee, A., Garg, A. and Sen, M.R. (2010). Antimicrobial susceptibility of

- pseudomonas aeruginosa isolated from wound infections. *Indian Journal Dermatology*; 51:2860-2880.
- Arora, D.R. and Arora, B. (2008). *A Text Book of Microbiology*. Third Edition: 653
- Bansal, E., Garg, A., Bhatia, S., Attri, A.K. and Chander, J. (2008). Spectrum of microbial flora in diabetic foot ulcers. *Indian Journal of Pathological Microbiology*; 51:204-208.
- Chincholikar, D.A. and Pal, R.B. (2002). Study of fungal and bacterial infections in diabetic foot. *Indian Journal of Pathology and Microbiology*; 45:15-22
- Eckhard, M., Lengler, A., Liersch, J., Bretzel, R.G. and Mayser, P. (2007). Fungal foot infections in patients with diabetes mellitus, results of two independent investigations. *Mycoses*; 50(2): 14-19.
- Frykberg, R.G. (2000). Diabetic foot ulcers: Current concepts. *Journal of Foot and Ankle Surgery*; 37(5): 440-446.
- Gupta, A.K., Humke S. (2000). Prevalence and management of onychomycosis in diabetic patients. *European Journal of Dermatology*; 10(5):379-384.
- Heald, A.H., O'Halloran, D.J. and Richards, K., Webb, F., Jenkins, S., Hollis, S., Denning, D.W. and Young, R.J. (2001). Fungal infection of the diabetic foot: two distinct syndromes. *Diabetes Medicine*; 18(7): 567-572.
- Hencevic, J. and Coce, F. (2002). *Diabetic Foot*. Zagreb: Medicinska Naklada.
- Hohaus, K., Vennewald, I. and Wollina, U. (2003). Deep mycosis caused by *Trichophyton mentagrophytes* in a diabetic patient. *Mycoses*; 46: 337-339.
- Kahn, R. (2007). Consensus development conference on diabetic foot wound care. *Diabetes Care*; 22(8): 1354-1360.
- Karel, B. and Phil R. (2005). *Diabetes Voice*; 50(1):14.
- Khanolkar, M.P., Bain, S.C. and Stephens, J.W. (2008). The diabetic foot. *Quarterly Journal of Medicine*; 101:685-695.
- Malcolm, D, Richardson W. (2012). Introduction, Diagnosis and management of mycoses: 5.
- Mayfield, J.A; G.E. Reiber, L.J. Sanders, D. Janise, and L.M. Pogachi (2004). "Preventive foot care in people with diabetes," *Diabetes Care*; 21(12): 2161-2177.
- Mehta, M., Dutta, P., and Gupta, V. (2010). Bacterial isolate from burn wound infections and their antibiograms; An eight-year study. *Indian Journal Plastic Surgery*; 40:25
- Nair, S., Peter, S., Sasidharan, A., Sistla, S. and Unni, A.K. (2007). Incidence of mycotic infections in diabetic foot tissue. *Journal of Culture Collection*; 5: 85-89.
- Nwachukwu, N.C., Orji, F.A., and Okike U.M. (2009) Antibiotic susceptibility of bacteria isolate from surgical wounds in Abia State University Teaching Hospital (ABSUTH), Aba Nigeria, *Research Journal of Medicine and Medical Sciences*;4(2):575-579.
- Ogbera, A.O., Adedokun, A., Fasanmade, O.A., Ohwovoriole, A.E. and Ajani, M. (2005). The foot at Risk in Nigerians with Diabetes mellitus: *The Nigerian Scenario. Internal. Journal of Endocrinology and Metabolism*; 4: 165-173.
- Romano, C., Massai, L., Asta, F. and Signorini, A.M. (2001). Prevalence of dermatophytic skin and nail infections in diabetic patients. *Mycoses*; 44: 83-86.
- Saunte, D.M.L., Holgersen, J.B. and Haedersdal, M. (2006). Prevalence of toe nail onychomycosis in diabetic patients. *Acta Dermatophyte of Venereology*; 86: 42-58.
- Shittu, A.O., Kolawole, D.O and Oyedapo, E.A.R (2002). Wound infections in two health institutions in Ile-ife, Nigeria. Result of a Cohort Study. *Osteomyelitis and wound Management*; 49(5):52-57.
- Spelman, D. and Spelman, W. (2002). Hospital acquired infections. *MJA Practice Essential Infectious Disease*; 176: 286-291.
- Taj-aldeen, S.J., Gent, J., Albozom, I., Buzina, W., Cano, J.F. and Guarro, J. (2006). Gangrenous necrosis of the diabetic foot caused by *Fusarium acutatum*. *Medical Mycology*; 44: 547-52.
- Van Houtum, W.H. (2008). 5th International symposium on the diabetes. *Metabolic Research Review*; 24 (1): S1-S2.
- Viswanathan, V., Jasmine, J.J., Snehaltha, C. and Ramachandran, A. (2002). "Prevalence of pathogens in diabetic foot infection in India types 2 diabetic patients," *Journal of Association of Physicians of India*

50(8):1013-1016.
Yah, S.C., Haruna, T., Enabulele, I.O and Jacob,
J. (2010). Bacterial Adherence; The role of

Serum and wound fluid. *Sudanese Journal of
Public Health*; 5(1): 15-21.

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