Original Research Paper

Volume : 5 | Issue : 9 | September 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Study of Fungal Infection in Diabetic Foot Ulcer

Medical Science

KEYWORDS : Diabetic ulcer, Fungal infection, Chronic ulcer, Foot ulcers.

Vikram Kala	Assistant professor, Department of medicine, BPS GMC for Women's Sonipat Haryana.
Prerna Panjeta	Assistant professor, Department of Biochemistry, BPS GMC for Women's Sonipat Haryana.
Ashutosh Kumar	Demonstrator, Department of Biochemistry, BPS GMC for Women's Sonipat Haryana.
Kashmeer khan	Sr. Demonstrator, Department of biochemistry, JLN Medical College, Ajmer Rajasthan.
Manish Sharma	Junior resident, Department of Medicine, BPS GMC for Women's Sonipat Haryana.

ABSTRACT

Background: *The objective of this study is to determine the prevalence of fungal infection in diabetic foot ulcers and to study the factors influencing fungal positivity.*

Methods: *A total of 100 diabetic foot ulcer patients admitted or who visited on outpatient basis to our hospital over a period of 1 year from May 2014 to April 2015. Fungal isolation from the ulcer was done by 10% KOH study, Gram stain, SDA culture and slide culture methods. The outcome of the disease was studied based on ulcer progression; wound healing & tissue loss/ amputation.*

Results: *In present study polymicrobial flora in diabetic foot ulcer was seen (137 organisms in 100 subjects), predominantly with entero-bacteriaceae and pseudomonas. Prevalence of coexisting fungal infection in the present study was 19% of which candida species was the commonest. Fungal infection was more commonly seen in long standing (4-5 months) non-healing ulcer on antibiotic therapy.*

Conclusion: *The present study signifies the need of mycological evaluation of a non-healing diabetic ulcer of a longer duration, with poor progression despite antibacterial therapy and foot care, and introduction of prudent antifungal treatment for proved fungal infection in diabetic foot ulcer and thus, to consider fungal infection as a significant risk factor in diabetic foot ulcer.*

INTRODUCTION

Diabetes is one of the major problems of this generation with worldwide distribution. It is a disease that involves many organs and systems of the body, notably the eyes, the kidney, the blood vessels and peripheral nerves. The worldwide prevalence of DM has risen dramatically over the past 2 decades, from 30 million cases in 1985 to 177 million in 2000. Based on current trends, > 360 million will have diabetes by 2030[2].

India today leads the world with the largest number of diabetic patients in any given country. It is said that presently 33 million Indians are diabetic and these numbers are likely to increase to 57.2 million by the year 2025[3]. This will be 1/6th of the world's total diabetic. Current prevalence rate is 12.1% in the urban Indian adult population. There is evidence that the prevalence is increasing in rural population also. India is already the Diabetic capital of the world.

Out of the 2 types, the incidence of type-2 DM is very high amongst Indians with 94-98% of Indians diabetics belonging to this group[3]. The number of detected cases of DM reflects only the tip of the iceberg, because of the large number of undiagnosed patients. The morbidity and mortality increases each year due to its complications, which are common in age group of 40-50 years affecting both sexes equally. The complications are more prevalent among the lower socioeconomic status because of negligence, illiteracy, poverty and many other concomitant factors.

Foot ulcer is one of the several chronic complications of uncontrolled diabetes. Approximately 15% of individuals with DM develop a foot ulcer (great toe or meta-tarso phalangeal area are more common) [4]. Diabetes is the leading cause of non-traumatic lower extremity amputation in developing countries[5]. The risk of lower limb amputation is 15-46 times higher in diabetics than who are not. Almost 20 out of 100 uncontrolled diabetic Indians undergo lower limb amputation[6].

The reason for foot being the commonest site for complications in diabetics, are that foot is the most vulnerable part of the body for injury and also the most neglected, secondly it is the site of preference for neuropathy and ischemia[6]. Diabetic ulcers occur due to 3 factors:

Trophic changes resulting from peripheral neuritis

Atheroma of the arteries resulting in ischemia.

Excess of sugar in the tissues, which lower resistance to infection, including fungi[7, 8].

The present study aims at determining the prevalence of fungal infection in diabetic foot ulcers and to study the factors influencing fungal positivity. It also aims to study the outcome of therapy with respect to response to anti-fungal therapy, wound healing process, tissue loss/amputation and patient satisfaction.

METHODS

A total of 100 cases attending outdoor clinics or admitted to Bhagat Phool Singh (BPS) Government medical college for women, Khanpur Kalan, Sonipat (Haryana), for the treatment of diabetic foot ulcer during the period of study from May 2014 to April 2015 were included in the study. This was an observational descriptive study.

Inclusion criteria included:

All patients admitted in our hospital and patients attending outdoor clinics for diabetic foot ulcer management.

Non-healing ulcer of duration > 2 months on antibiotic and wound care.

Exclusion criteria:
Ulcer on foot other than diabetic like secondary to venous disorders, arterial diseases.

Severely infected diabetic ulcer with systemic infection and septicemia.

Patient already on antifungal treatment for a diagnosed fungal infection

Patient allergic/contraindicated for antifungal treatment.

Pregnant and lactating women.

Written informed consent was taken from all the subjects along with clearance from the ethics committee. A Performa was developed to record the medical history, examination details and investigations reports. Medical history was taken for all subjects. Details regarding type for diabetes, its duration, its treatment, compliance and personal habits were recorded. Meticulous examination was done including description of ulcers (site, size, shape, grade, classification) as per the Performa of the study and tests performed to check for neuropathy and ischemia. Necessary investigation reports required for the study were documented in the performa. Investigation required were recent fasting and post prandial plasma glucose levels, serum creatinine, HbA1c, PUS c/S, X ray foot to rule out osteomyelitis, Arterial / Venous Doppler if vascular pathology is suspected.

Sample collection: ulcer was cleaned with povidone iodine solution and sterile normal saline before collecting the samples. Tissue sample for the study was taken from the depth of the ulcer including the edges, consisting mostly the granulation tissue and the necrotic slough from the ulcer bed. Average size of the tissue block collected measured around 0.5*0.5 cm^9.

Tissue samples were collected in plastic bottles (autoclavable) containing approx. 4-5 ml of normal saline. Tissue samples were collected from the ulcers under aseptic precautions.

The tissue blocks were triturated and processed as per standard protocols.[10, 11,12]

Preliminary examination

KOH 10% Preparation

Gram's staining

Cultural procedure:

Triturated tissue sample are inoculated on to 4 tubes containing SDA agar with antibiotics – chloramphenicol. One pair of culture tubes is then incubated at room temp. And other pair at 37 deg. Growth on culture is examined twice in a week up to 4-6 weeks before it was declared negative. Sensitivity for anti-fungal agents was done in present study.

Lacto phenol cotton blue (LPCB) mounting: the growth seen on the culture media are mounted using this LPCB mount it helps in the microscopic identification of the fungal elements

Slide culture: following LPCB mount slide culture is done to confirm the intact morphology of the fungal isolate.

RESULTS AND DISCUSSION
The results of the study showed that the majority of the patients were in age group of 51-70 consisting of 56% of the study cases.71% were male and 29% were females. 44% had history of spontaneous onset while 56% had history of trauma. The mean duration of the ulcers in the study was 3.45 months. The Duration of ulcer in various patients was shown in Table 1.

TABLE 1: Duration of Ulcer in Months in patients.

Duration of ulcer (Months)	Number of pt.	No. Of patient with positive fungal isolation	%	P value
2-3	43	5	11.63	0.618
3-4	34	5	14.71	0.523
4-5	11	6	54.55	0.003
5-6	9	3	33.33	0.273
>6	3	0	0	-
Total	100	19	19	-

The mean duration of diabetes in patients in our study was 7.65 years. The Duration of diabetes in various patients was shown in Table 2.

TABLE 2: Duration of Diabetes in patients

Duration of diabetes(year)	Number of pt.	%
<5	42	42
5-10	36	36
10-15	14	14
15-20	5	5
>20	3	3
Total	100	100

In 100 patients studied 137 organisms (118 aerobic bacteria and 19 fungi) were isolated. This represents an average of 1.37 organisms per case. Specimen from 8 ulcers was sterile. Among 92 specimen showing some growth, only bacteria were isolated in 73 cases, whereas mixed infections of both bacteria and fungi was found in 13 cases. More than 1 bacterium was grown in 18 specimens. Poly microbial flora was seen in 31 specimens, whereas mono microbial flora in 61 cases. Pus cells were seen with all bacterial isolates. Distribution of various microbes in ulcer was shown in Table 3.

TABLE 3: Distribution of various microbes in the ulcers.

Bacterial isolation	Total no. Of patients	%
Pseudomonas	29	27.88
Staph aureus	19	18.27
Enterococcus	15	14.42
Klebsiella	11	10.58
E. coli	7	6.7
Enterobacter	7	6.7
Proteus	6	5.77
Acinetobacter	5	4.8
Citrobacter	5	4.8
>1 bacteria	18	17.3
No growth	14	14

Out of 100 subjects 19 of them had fungus grown in the tissue specimen obtained from their foot ulcers. Out of 19, 6 (31.58%)

Volume : 5 | Issue : 9 | September 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

of them were isolated growths as proved by pus c/s. The remaining 13 (68.42%) were co-existed with at least 1 bacterium in the ulcer. Pseudomonas (53.85%) was the commonest bacteria co existing with fungal infection in diabetic ulcers includes in our study. Second commonest was staph aureus accounting for 30.77 % of mixed infection.

Table 4: Bacterial isolation in patient studies and fungal positive patients

Bacterial isolation	Total no. Of patients	No. Of patients with positive isolation	%	P value
Pseudomonas	29	7	24.14	0.481
Staphaures	19	4	21.05	0.819
Enterococcus	15	1	6.67	0.223
Citrobactor	5	-	-	-
Protues	6	-	-	-
Klebsiella	11	1	9.09	0.402
E. coli	7	-	-	-
Actinobactor	5	-	-	-
Enterocbactor	7	-	-	-
No growth	14	6	42.86	0.023
>1 bacterium	18	-	-	-
Total	100	19	19	-

Table 5: Intervention on follow up in patients studied and fungal isolation

Intervention required on follow up	Total no. Of patients	Number of pt. With positive fungal isolation	%	P value
Only dressing	28	6	21.43	0.743
Sec. Suturing	24	4	16	0.702
SSG	24	8	33.33	0.074
Disarticulation or amputation	23	1	4.35	0.073
Total	100	19	-	-

List of all fungus isolated in our study:
Candida: 5 no.

Trichosporon: 1 no.

Aspergillus: 4 no.

Fusarium spp.: 3 no.

Trichophyton spp :3 no.

Penicillium: 2 no.

Acr emonium: 1 no.

Out of the 19 fungi isolated in our study the commonest fungal growth was candida species, accounting for 26.32% of total growth. This was followed secondly by Aspergillus species. Accounting for 21.05% of total growth. Based on morphology, all the fungal isolates obtained in our study were classified as yeasts and molds. Yeast were 6 in no. (31.58%) and molds were 13(68.42).

CONCLUSION:
In the present study, randomly 100 cases of diabetic foot ulcers were studied for the prevalence of fungal infection and the factors influencing its co-existence in the ulcer. Majority of patients in present study were males (71%) with the mean age of 58.2 yrs. All cases were of type 2 DM on treatment, with majority of them having DM for more than 5 years. In present study the ulcers included were of chronic non healing type, of more than 2 months' duration.19 cases showed positive for fungal infection, commonest fungi isolated were candida (26.3), followed by aspergillus (21.1).

The results obtained in the study were analyzed the various factors influencing fungal infection in diabetic foot ulcers were studied. Fungal isolation was more common in traumatic ulcers at 23.21% compared to 13.64% in ulcers with history of spontaneous onset. On comparing the grading of ulcers with fungal isolation higher grade ulcers did not show any predisposition to fungal isolation. The fungal isolation rate was 20.51% in Grade 2 ulcers, 20.93% in Grade 3 Ulcers and 11.11% in Grade 4ulcers. There was no statistically significant difference.

Depending on duration of ulcer long standing ulcers (>4 months) had more frequent isolation of fungal elements with a **P value of 0.003**, which is statistically significant. Table 1 shows correlation of ulcer duration and fungal isolation.

However, the duration of diabetes seems to have no statistically significant role in determining the incidence of fungal infection as analysis of the results revealed that 19.05% of fungal isolates was from patients with diabetes <5 years, 19.44 % of fungal isolates was from patients with diabetes 5-10 years. 21.43% of fungal isolates was from patients with diabetes 10-15 years and 20% of fungal isolates was from patients with diabetes >15 years. These were not statistically significant. Long Term blood sugar control also did not have any statistically significant difference in determining the incidence of fungal infection as interpreted by comparing incidence of fungal infection in patients with varying HbA1c Levels.

However, on detailed study of the microbiological flora in each ulcer and correlating it with fungal isolation it revealed that there was significant association between fungal infection and ulcers having no growth on pus c/s. This was statistically significant with a **P value of 0.023.** Thus it can be assumed that isolated fungal infection is more common owing to the long term antibiotic therapy inhibiting any bacterial growth in the ulcer. This data is depicted in Table 4. Amongst bacteria pseudomonas and staph aureus commonly existed with fungal infection in diabetic ulcer. The pattern of Microbiological flora isolated was comparable to other studies[13, 14].

The response of the fungal ulcers to various modalities of treatment is depicted in Table 5. Anti-fungal therapy was started in these patients and progression of each ulcer was studied from time of inclusion, both during hospital stay and after discharge and was analyzed based on the healing of ulcer. In present study, it was noticed that ulcers with fungal infection on anti-fungal therapy had a good prognosis with significantly less amount of tissue loss. Our results were comparable to other studies[15]. Hence treating fungal infection in diabetic foot ulcers significantly lead to limb salvage.

Thus the present study throws light on various aspects of fungal infections in diabetic ulcers. The clinical implications of the study are:

All long term Diabetic foot ulcers need to be investigated for fungal elements.

Diabetic Ulcers with no bacterial growth need to be investi-

gated for fungal growth.

Unnecessary use of antibiotics should be restricted.

Prompt treatment of fungal ulcers with antifungals will improve limb salvage rates.

REFERENCES

1. Alvin C Powers. Chapter 338. Diabetes Mellitus. Harrison's Principle of Internal Medicine- Vol 2. 17th Edn;Pg 2275.
2. Sahay BK. API Textbook of Medicine – 8th Edn. Vol 2.Sec 18-Diabetology; Pg 1042
3. Sridhar GR, Rao PV and Ahuja MMS. RSSDI Textbook of Diabetes (Eds Ahuja, M.M.S. et al.), RSSDI, Hyderabad, 2002, pg 95-112.
4. Ravisekhar Gadepalli, Msc , Benu Dhawan, MD, Vishnubhatla Sreenivas, PHD, Arti Kapil, MD, A.C. Ammini, DM and Rama Chaudhry, MD; A Clinicomicrobiological Study of Diabetic foot Ulcers in an Indian Tertiary Care Hospital. Diabetes Care 29:1727-1732,2006
5. Lavery LA, Ashry HR, Van Houtum W,Pugh JA, Harkless LB,Basu S. Variation in the incidence and proportion of diabetes-related amputations in minorities. Diabetes Care 1996;19:48-52.
6. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, Lefrock JL, Lew DP, Mader JT, Norden C, Tan JS. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2004 Oct 1; 39(7):885-910.
7. Seema Nair, Sam peter et al. Incidence of Mycotic infections in Diabetic Foot tissue. J of Culture collections: Vol 5:2006-2007;85-89.
8. Chincholikar DA, Pal RB. Study of fungal and bacteriological infections of the diabetic foot. Indian J Pathol Microbiol 2002; 45:15-22.
9. Vishwanathan V, Jasmine JJ, Snehalatha C et al. prevalence of pathogens in diabetic foot infection in South India type 2 diabetic patients. J Assoc Physicians India 2002;50:1013-6
10. Ananthanarayan R, Paniker CKJ. Textbook of Microbiology. 6th edition. Medical Mycology. Chap 65;Pg 564-579
11. Silver-Hunter and Cooper: In Linnette et al: Manual of Medical Microbiology, 3rd ed,1980, Washington D.C., American society of Microbiology.
12. Leake JL,Dowd SE, Wolcott RD,Zischkau Am. Identification of yeast in chronic wounds using new pathogen-detection technologies.; Texas Tech University/ Howard Hughes Medical Institute Program, Lubbock, Texas, USA.
13. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. India J Pathol Microbiol 2008;51:204-8
14. Emiija Mlinaric-Missoni, Smilja Kaenic, Milan Vukelic. Candida Infections of Diabetic Foot Ulcer-Original Scientific paper. April 11, 2005. Diabetologia Croatica 34-1,2005
15. Richard L Simmons, Richard J Howard. Surgical infection diseases. Second edn; pg 563-572.