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Study of Fungal Isolates from Deep Tissue Samples and their Role in A Diabetic Foot Patients

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ABSTRACT
Diabetic foot ulcers contribute a major cause of non- traumatic amputations. Diabetes being the major cause of immunosuppression may facilitate fungal infections in diabetic foot patients. Diabetes status of patients who presented with foot ulcers were confirmed by using standard protocol. Deep tissue samples were collected and processed for both bacterial and fungal cul-tures as per the standard methods. Apart from bacterial isolates, fungus was isolated from 25% of the patients. Candida albicans being the most common isolate followed by Candida tropical is. The longer the duration of ulcer higher the chances of fungal isolation seen. Grade II and Grade III ulcer showed more number of fungal isolates compared to Grade I and Grade 0 ulcers. Hence while treating Diabetic foot ulcers chances of fungal infections should be considered.

INTRODUCTION

The incidence of diabetes is increasing around the world, including India. India has been called the "Diabetes capital of the world" and "every fifth diabetic in the world is Indian"[1]. Diabetes is expected to increase from 2.8% in 2000 to 4.4%by 2030 in all age groups worldwide[2]. The lifetime risk of having diabetic foot ulcer in a person is between19-34%[3].

In India 15% of Diabetic patients are expected to have diabetic foot ulcer during their life time. Diabetic foot ulcers contribute to approximately 80% of all non-traumatic amputations[7]. Globally the long term outcome of patients was found to be poor even after surgical and antibacterial therapy for infected diabetic lower limb wounds[8].

Diabetics with ulcers commonly experience infection with gram-positive organisms such as *Staphylococcus aureus*, *Enterococcus* and gram-negative organisms like *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella species*, *Proteus species*, etc., and anaerobes[4].

The prevalence of fungal infections Like candidiasis has been widely studied, particularly in Diabetic patients. Susceptibility to fungal infections is high in Diabetic patients compared to non diabetic patients[6,5].

Fungi rarely cause infection in healthy host. Fungi can cause infection only when there is breach in the protective barrier or any debilitating conditions can favour fungal entry and growth. They tend to colonise and multiply in the region where they gained access. The amount of inoculum the level of the tissue destruction the ability of the fungi to multiply in that tissue and the immune status of the host are the different factors on which the severity of the disease is dependent[10].

The countries with low socio economic status seems to have high rate of fungal infections compared to developed countries. The factors like living in a crowded atmosphere with consistent human to human contact, suboptimal hygiene, poor environmental health practices and other unhygienic conditions may enhance the chances of fungal infections[9].

India being a developing country have people with low socio economic status and these people due to lack of awareness ends up in serious infections because of inadequate medical attention. The chances of these patients ending up with fungal infections is very high as Diabetes is the major cause of immunosuppression.

MATERIALS METHODS

Study design: The spectrum of fungi isolated from diabetic foot ulcer was not studied extensively and the chances of ignoring fungal infections while treating the patients are very high. Hence a cross sectional study was done to emphasise the importance of treating fungus isolated from the deep tissue sample of a diabetic foot ulcers.

Participants/subjects: A study was carried out on all male and female patients presented with diabetic foot ulcer in the department of General surgery for a period of 1 year. The total number of patients studied were 60.

Inclusion criteria: All diabetic foot patients who attended the Surgery OP.

Exclusion criteria: The patients who were on antifungals, chemotherapy, immunosuppressants and who had skin grafting on the feet were excluded from the study.

Procedure/methodology: Biochemical profile (FBS, PPBS, HbA1C) was done to asses the status of Diabetes mellitus[12]. Two tissue samples were collected from the bed of the diabetic foot ulcer after debriding the slough and necrotic tissue and the sample was transfered in a sterile container with normal saline[11-24]. The sample was processed for microscopy and fungal culture in the Microbiology laboratory[25]. One sample was used for microscopy with the help of 10 % KOH while the other was inoculated in 2 sets of Sabourauds Dextrose agar with Chloramphenicol for fungal culture . One is incubated at room temperature and the other at 37 °C for one month[11]. The media was observed for growth daily for one week and then once in 2 days for rest of the period. Any growth on the culture media was identified by macroscopic and microscopic morphology such as Lactophenol cotton blue and slide culture.

Presumptive identification of Candida species were made by appreciating morphology on corn meal agar, by doing germ tube test and inoculating on HiCromeTM Candida Differential Agar Base, Modified M 1456A[25].

RESULTS

Out of 60 patients studied in our hospital 51 were males and 9 were females.The youngest was 29 years old and oldest was 82 years old. Prevalence of Fungal infection was seen in 15 patients. Among 15 patients 10 were males and 5 were females.

The highest incidence of fungal infection is seen in the age group above 61years where as no cases were reported in patients from age 20-30 years.

The highest incidence of fungal infections were seen in patients with more than 20 years duration of diabetes where as lowest incidence was seen patients with less than 5 years duration of diabetes.

The incidence of fungal isolates is increasing with duration of ulcer. No fungus was isolated from patients with less than a month duration of ulcer whereas

Table 1: Age distribution of the samples

Age in years	20-30	31-40	41-50	51-60	>61
Number of patients	3	10	12	15	20
Patients with fungus isolation	0	2	3	4	6

Table 2: Duration of Diabetes

Duration of Diabetes in years	<5 years	05-10 years	11-15 years	16-20 years	>20 years
Number of patients	7	11	11	12	19
Patients with fungus isolation	1	2	3	3	6

Table 3: Duration of ulcer

Duration in months	<1	02-03	03-04	04-05	05-06	>06
Number of patients	5	8	16	18	13	0
Patients with fungus isolation	0	1	3	6	5	0

Table 4: Grade of ulcer

Grade of ulcer	0	I	II	III	IV	V
Number of patients	0	12	20	17	0	0
Patients with fungus isolation	0	2	7	6	0	0

highest incidence of fungal isolates were seen in patients having more than 5 months duration of ulcer. But no case of ulcer with more than 6 months duration was reported.

The incidence of fungal isolates increased with the grading of ulcer.

Among 60 patients 35 were smokers and 22 consumed alcohol.

Out of 60, 26 were on oral hypoglycemic agents out of which 6 had fungal infection and 34 were on Insulin out of which 9 had fungal infection.

All of them had Type II Diabetes mellitus and associated co morbid conditions like Hypertension was seen in 20 patients out of which 3 had fungal infections, Chronic renal failure was seen in 2 patients and both of them had fungal infection, Hypothyroid is seen in 2 patients and no fungus isolated from them.

Predominantly Candida species were isolated in 14 patients and Aspergillus is seen in 1 patient.

Out of 14 patients with Candida infection 9 patients were having Candida albicans, 4 patients were having Candida tropicalis and 1 patient was having Candida krusi. And 1 patient showed growth of Aspergillus.

` Surgical debridement was done in all 15 patients with fungal infection and were treated with broad spectrum antibiotics and antifungals. Amputation was needed in one patient. No mortality was observed. All patients recovered after treatment.

DISCUSSIONS

WHO identified Diabetes as one of four priority non-communicable diseases (NCDs) as these diseases are responsible for 71% of all deaths globally (WHO). And these diseases may attribute to premature deaths out of which 85% of them occur in Low and Middle income countries.(WHO). People who are living with diabetes are 20.3 times more likely to have lower limb amputation than people without Diabetes[14]. Diabetic foot amputation remains an unpleasant impact on patient's life more than other complications.

The present study was carried out to evaluate the fungal infections associated with diabetic foot ulcer. Out of total 60 patients under study 85 % were males and 15% were females which shows that males were more commonly effected with diabetic foot ulcer than females. similar to studies done Seth *et al.*[17] Eckhard *et al.*[18]. And most of the authors agree that incidence of Diabetic foot ulcer is high in males compared to females.

Our study showed that the incidence of Diabetic foot ulcer is increasing with age as well as the fungal infections associated with diabetic foot ulcer is also increasing with age. The same is seen with many studies Fata *et al*.[15] Al-Maskari *et al.*[19] Imran *et al*.[20] Unnikrishnan *et al*.[21]

In the present study Incidence of fungal infections is more in patients with prolong duration of ulcer that is more than 1 month. These chronic wounds which are believed to be colonized with polymicrobial communities keeps stimulating inflammatory reaction. And these wounds when covered with dressing the deeper tissue which are always under moisture try to attract the growth of fungus. The same was explained in a study done by Kareliya *et al.*[22] Dawood *et al*.[27]

Deeper the ulcer more the chances of fungal infections. We have noticed that 46% of fungal infections are with Grade II ulcers and 40% are with Grade III ulcers. The same was demonstrated in different studies done by Anjana Gopi *et al*.[16] Anand *et al.*[23] Kareliya,[22] Gopi *et al*.[11]. And the study revealed that 25 % of the individuals were having fungal infections similar to the studies done by Saba Fata *et al.*[15] Anjana Gopi *et al.*[16] Gopi *et al*[11].

Candida is the most common fungi isolated in our study. Different species of Candida were isolated among which Candida albicans is the most common followed by Candida tropicalis. Studies done by Kalshetti *et al.*, Saba *et al.*[15] Anjana Gopi *et al.* [16] Shows similar results.

CONCLUSION

The present study emphasises importance of mycological evaluation in diabetic foot patients as the chances of fungal isolation is increasing with duration of ulcer and with the depth of an ulcer. Candida albicans is the most common fungi isolated followed by Candida tropicalis.

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