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A study of fungal infections of diabetic foot in tertiary care hospital of U.P

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Abstract
Objective: *To study the fungal infections of diabetic foot in tertiary care hospital of U.P.*
Methods: *This was a cross-sectional study. Diabetic patients (70) with foot ulceration were included in the study. Samples were obtained from the depth of the wound (taking aseptic precautions) after debridement .Samples were transported to the microbiology laboratory within an hour in sterile containers.*
Results: *More than one third of patients were between 60-69 years (48.6%) of age. Majority of patients were males (78.6%).KOH was positive among 31.4% patients. Fungal positive was 21.4% patients. Gram positive bacteria was seen in 36.4% and gram positive was in 63.6%. Among the gram positive, Enterococcus faecalis was most prevalent (75%) and among the gram negative Pseudomonas spp. was found to be most prevalent (50%). Candida albicans was most predominant (73.3%) and Aspergillus fumigatus was least common (33.3%).*
Conclusion: *Due to hyperglycemic environment and suppressed immunity, diabetic patients are more prone to infections. Fungal infections in diabetic patients if not treated in-time leads to the fatal complications such as foot amputation.*
Keywords: *Fungal infections, Diabetic foot, Prevalence.*

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from either inadequate insulin production, reduced tissue sensitivity to insulin or both. Chronic hyperglycaemia leads to diabetic complications including peripheral neuropathy, peripheral vascular disease, increased risk of infection and poor wound healing. The diabetic foot may be defined as a group of syndromes in which neuropathy, ischaemia, and infection lead to tissue breakdown resulting in morbidity and possible amputation (Markakis et al, 2016).[1]

Diabetic foot infections are a well-recognised risk factor for hospitalisation and amputation. According to a recent meta-analysis one in every 30 hospitalised patients at any given time is affected by a diabetic foot infection (Lazzarini et al, 2015)[2]. Additionally, patients with diabetes who develop an infection have been reported to have a 155-fold increased risk of amputation compared to those who do not. Nearly all diabetic

foot infections originate in a diabetic foot ulcer (DFU) and the prevalence of these infections in DFUs have been reported to range between 25–60%. Although the critical nature and prevalence of infected DFUs are well appreciated, the development of these infections in the first place has received less attention (Jia et al, 2017)[3].

Clinicians are faced with two common appearances of fungal infections of the foot: tineapedis (athlete's foot) and onychomycosis (nail infection). Within the general population, tineapedis and onychomycosis both have a prevalence of about 15%–20% and often coexist (Gupta et al, 1998; Thomas, 2010)[4,5]. The most common fungi that cause tineapedis are Trichophyton rubrum (80%) and Trichophyton interdigitale (15%), and less commonly Epidermophyton floccosum and Microsporum (British Infection Association)[6].

Hence, this study was under taken to study the fungal infections of diabetic foot in tertiary care hospital of U.P.

Material and Methods

This was a cross-sectional study design. The present study was conducted in the Department of Microbiology, Rama Medical College &Hospital, Kanpur-U.P. Diabetic patients with foot ulceration were included in the study. Patients who already underwent skin grafting on the feet were excluded from the study. Patients already treated with anti-fungal therapy, chemotherapy, immune-suppressants, radiotherapy and corticosteroids were excluded from the study. The study was approved by the ethical committee of the hospital. The inform consent was taken from each patient before enrolling in the study.

Sample Collection and analysis

Samples were obtained from the depth of the wound (taking aseptic precautions) after debridement. Samples were transported to the microbiology laboratory within an hour in sterile containers.

The necrotic areas of the tissues were mounted on KOH and also inoculated into Sabouraud's Dextrose Agar (SDA) (HiMedia Ltd, Mumbai). Specimens for bacteriological study was cultured in the following agar media: sheep blood, chocolate, and MacConkey agar. The fungal samples were incubated at 37 °C for 1 weak and will be evaluated daily for growth of fungal microorganism.

The colonies were identified on the basis of their macroscopic and microscopic (slide culture) features. Yeast samples were cultured in Chrom agar (HiMedia, India) for isolation and identification of Candida spp.

Analysis

The results are presented in proportions and mean±SD. The chi-square test was used to compare the categorical variables. Unpaired t-test was used to compare the biochemical parameters. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results

More than one third of patients were between 60-69 years (48.6%) of age followed by 70-79 (32.9%) and 40-59 (18.6%) years. Majority of patients were males (78.6%). Majority of patients were type-2 diabetic (75.7%).Duration of diabetes was 5-10 and 11-15 years in 30% patients (Table-1).

KOH was positive among 31.4% patients. Fungal positive was 21.4% patients (Fig.1).Fungal infection was higher among older patients, however, the association was insignificant (p>0.05). Fungal infection was higher among male patients (25.5%) than females (6.7%), however, the association was insignificant (p>0.05). Fungal infection was higher among type-1 diabetics, the association was significant (p=0.02). Fungal infection was higher among higher duration of diabetics, the association was significant (p=0.01) (Table-4).

Gram positive was seen in 36.4% and gram positive was in 63.6%. Among the gram negatives, *Enterococcus faecalis* was most prevalent (75%) and among the gram negative

Pseudomonas spp. was found to be most prevalent (50%) (Table-2).

Candida albicans was most predominant (73.3%) and *Aspergillus fumigates* was least common (33.3%) (Table-3).

Grade IV (33.3%) was most common and Grade I was least common (13.3%)(Fig.2).

Table-1: Distribution of patients according to basic profile

Basic profile	No. (n=70)	%
Age in years		
40-59	13	18.6
60-69	34	48.6
70-79	23	32.9
Mean±SD (Range)	55.14±13.12 (40-76)	
Gender		
Male	55	78.6
Female	15	21.4
Type of diabetes		
Type-1 diabetes	17	24.3
Type-2 diabetes	53	75.7
Duration of diabetes in years		
<5	15	21.4
5-10	21	30.0
11-15	21	30.0
>15	13	18.6
Mean±SD (Range)	10.36±5.41 (2-20)	

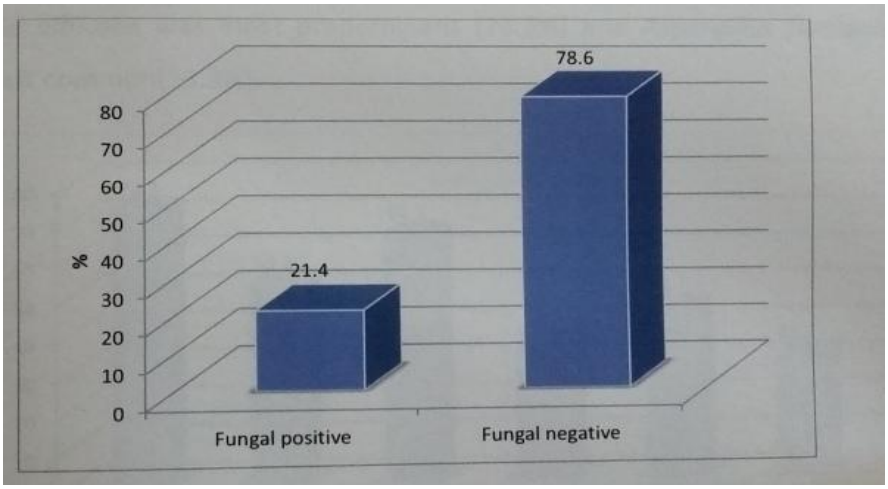

Fig.1: Distribution of patients according to KOH and Fungal culture tests

Table-2 : Distribution of gram staining bacteria

	No. (n=22)	%
Gram positive	**8**	**36.4**
Enterococcus spp.	4	50.0
Enterococcus faecalis	6	75.0
Staphylococcus aureus	5	62.5
Gram negative	**14**	**63.6**
Escherichia coli	5	35.7
Pseudomonas spp.	7	50.0
Klebsiella pneumonia	2	14.3
*Stenotrophomonas*spp.	1	7.1

Table-3: Distribution of patients according to Fungi isolated in Diabetic foot ulcer

Fungi isolated*	No. (n=15)	%
Candida albicans	11	73.3
Candida tropicalis	8	53.3
Candida glabrata	10	66.7
Trichophytonmentagrophytes	6	40.0
Trichophytonrubrum	7	46.7
Aspergillus fumigates	5	33.3

*Multiple response

Table-4: Comparison of fungal infection with various factors

Factors	No. of patients	Fungal positive		p-value[1]
		No.	%	
Age in years				
40-59	13	2	15.4	0.83
60-69	34	8	23.5	
70-79	23	5	21.7	
Gender				
Male	55	14	25.5	
Female	15	1	6.7	0.11
Type of diabetes				
Type-1 diabetes	17	7	41.2	0.02*
Type-2 diabetes	53	8	15.1	
Duration of diabetes in years				
<5	15	1	6.7	
5-10	21	3	14.3	0.01*
11-15	21	4	19.0	
>15	13	7	53.8	

[1]Chi-square test, *Significant

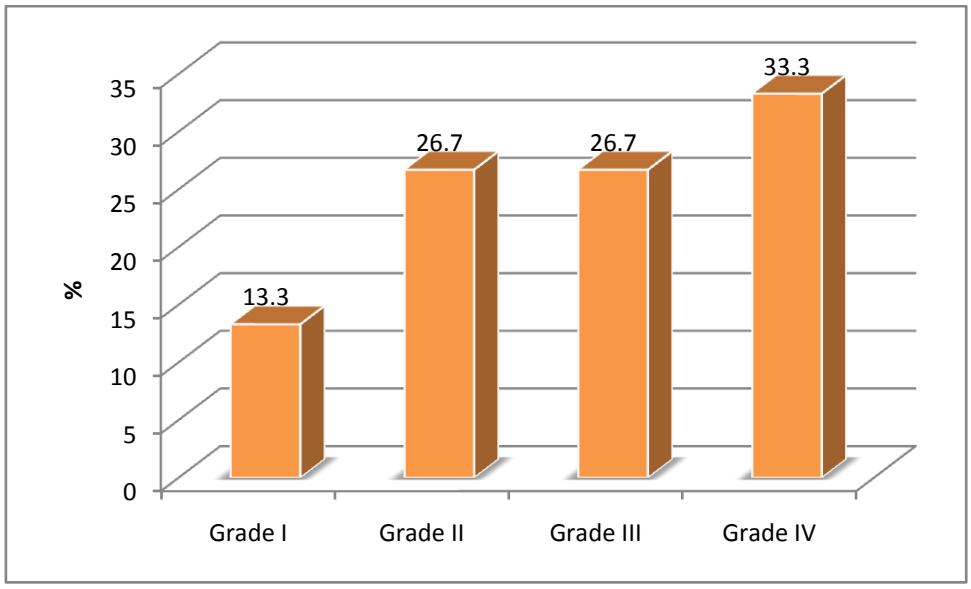

Fig. 2: Distribution of patients according to Wagner's grade

Discussion

Diabetes is one of the most common causes of multifocal peripheral neuropathy, which further gives rise to diabetic foot ulcers. Neuropathy in diabetic patients is manifested in the motor, autonomic and sensory components of the nervous system (Dyck et al, 2012; Clayton and Elasy, 2009).

Diabetic foot is one of the most feared complications of diabetes, which may result in

repeated hospitalizations leading to amputations. It is common affecting up to 30% of Indian patients with diabetes in their lifetime (Gupta, 2012). Another study conducted by Shahi et al (2012) in northern India claim that the prevalence of DFUs in patients with diabetes was 14.30%.

The present study was conducted in the Department of Microbiology, Rama Medical College & Hospital, Kanpur-U.P with the objective to study the fungal infections of diabetic foot and to isolate and identify the fungal infections.

In the present study, More than one third of patients were between 60-69 years (48.6%) of age followed by 70-79 (32.9%) and 40-59 (18.6%) years. In a study (Varsha et al, 2017), the age of patients ranged between 32-73 years. Several studied have reported that the incidence of diabetic foot increases with increasing age (Al-Maskari and El-Sadig, 2007; Al-Tawfiq and Johndrow, 2009). In another study (Singh and Singh, 2017),30 subjects were 40 years and below, 50 (41.6%) were within the 41-60 years age range while 40 (33.3%) were more than 60 years old.

In this study, majority of patients with diabetic foot ulcers were males (78.6%). This finding is in agreement with the studies conducted by Varsha et al (2017), Hayat et al (2011), Hena and Growther (2010), Sharma et al (2006) and Ahmed and EI-Tahawy (2000).

In the present study, majority of patients were type-2 diabetic (75.7%). In a study (Varsha et al, 2017), among the 80 patients 68 (85%) had type II mellitus and 12 (15%) had type I diabetes mellitus. In another study (Singh and Singh, 2017), out of 120 diabetics, 110 (91.7%) subjects had type 2 diabetes and only 10 (8.3%) had type 1 diabetes.

The duration of diabetes also plays a role in the formation of ulcer and the incidence of lower extremity amputation was more in patients who have diabetes for more than 5 years. Longer the duration of diabetes, higher are the chances of developing a non-healing ulcer. In the present study, duration of diabetes was 5-10 and 11-15 years in 30% patients. (Singh and Singh, 2017)reported that out of 120 patients, majority of patients who underwent amputation had diabetes more than 10 years ($p<0.05$). In another study (Abilash et al, 2015), 13% had diabetes for less than 5 years, 32% of them had from 5 to 10 years, 10% had from 11 to 15, 12% had from 16 to 20 years, and 33% had for more than 20 years. In another study (Singh and Singh, 2017), the duration of diabetes was equal to or less than 5 years in 5 (4.16%) subjects, 90 (75%) subjects had it for a period of between 6 - 10 years while only 25 (20.8%) had been diabetic for more than 10 years.

In this study, KOH was positive among 31.4% patients. In a study (Wijesuriya et al, 2014), 80% were KOH positive cases. This difference between the study might be due to different socio-economic status and environment.

In the present study, fungal positive was 21.4% patients. This finding is slightly higher than the study by Varhsa et al (2017) in which the total fungal positive cultures seen was 17.5%. Kannan et al (2015) and Chellan et al (2010) reported similar finding as in the present study. In another study (Abilash et al, 2015)[11], fungi were found in 27.2%.

In this study, *Candida albicans* was most predominant (73.3%). Similar finding was reported by Varsha et al (2017) in which the predominant *Candida* was the *Candida albicans*42.85%. The finding of this study was consistent with other studies also (Fata et al, 2011; Nair et al, 2006)[, 106]. In a study by Chincholikar and Pal (2002), swabs were collected from 105 diabetic foot ulcer patients, which revealed that the fungal isolates accounted for 20.8%. Among this yeasts were predominant accounting for 94.55% and moulds comprised 5.46%. *Candida tropicalis* (54.55%) were most common followed by *Candida albicans* (12.73%) among the yeasts.

In the present study, *Trichophyton mentagrophytes, Trichophyton rubrum and Aspergillus fumigatus* was in 40%, 46.7% and 33.3% respectively. Varhsa et al (2017) reported

that the other species of *Candida* isolated were *Candida tropicalis* (21.42%) and *Candida glabrata* (14.28%). In some other studies, species like *Candida parapsilosis, Candida guilliermondi, Candida tropicalis, Candida glabrata, Candida fumata, Candida kefur* had been reported (Bansal et al, 2008; Chincholikar and Pal, 2002; Heald et al, 2001). In another study by Abilasha et al (2015)[11], the most common Candida species was found to be *C albicans*[88.8%] followed by *C tropicalis* [11.11%] of the total fungal isolates substantiated by a study conducted by Nair et al (2006-2007)to assess the incidence of mycotic pathogens in diabetic foot ulcers.

In this study, Grade IV (33.3%) fig.(2), was most common and Grade I was least common (13.3%). None of the study had reported grades, hence comparison could not be made.

In this study, Fungal infection was higher among older patients, however, the association was insignificant (p>0.05). Amal and Noha (2015) found that fungal foot infection were highest among the age group 51-60 years.

In this study, fungal infection was higher among male patients (25.5%) than females (6.7%), however, the association was insignificant (p>0.05. Varsha et al (2017) reported that out of fungal positive cases, 9 (64.28%) were males and 5 (35.71%) were females.

In this study, fungal infection was higher among type-1 diabetics, the association was significant (p=0.02). Fungal infection was higher among higher duration of diabetics, the association was significant (p=0.01).

There are some limitations of this study. This study may not reflect all the aspects of management of diabetic foot ulcers, further, the study was among a small group of 70 patients in a short period . A larger study spanning over longer period and a bigger sample size is required to draw definitive conclusions.

Conclusion

Due to hyperglycemic conditions and suppressed immunity, diabetic patients are more prone to infections. Fungal infections in diabetic patients if not treated in-time leads to the fatal complications such as foot amputation.

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