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A STUDY ON PREVALENCE OF SUPERFICIAL MYCOSES IN DIABETIC PATIENTS IN TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

INTRODUCTION : Diabetes mellitus ,chronic metabolic dysregulation of glucose is a world wide problem of increasing importance. Cutaneous infection accounts for 20-50% of the skin manifestation among diabetic patients due to poor glycaemic control[1,2,3,4]. The fungal infections is the commonest cutaneous infection in diabetic patients.Moreover the prevalence of superficial mycoses namely onychomycosis and tinea pedis is 75% in diabetic patients especially in type 2.[5,6,7].The relative occurrence of the etiological agents of these superficial mycotic infections varies among countries[8]. Hence this study was conducted to know the prevalence and the causative agents in our region. . This causes considerable morbidity,disability and also leading cause for foot amputations [9]and hospital stay.[10,11,12]. Hence early detection of superficial mycoses especially tinea pedis and onychomycosis of toe nails , adequate dosage of antifungal agents with good glycaemic control can save the limb.

Aims & Objectives: To isolate and identify the casautive organisms of superficial mycoses in diabetic patients.

To study the prevalence of superficial mycoses in diabetes mellitus.

MATERIALS AND METHODS: A cross sectional study was conducted with 150 diabetic patients with superficial mycotic infection by collecting skin scrapings and nail clippings.Direct microscopy with KOH mount followed by inoculation onto SDA in duplicate set was done to be incubated at 25^{0}C and 37^{0}C.Sample was also inoculated onto DTM with antibiotics and chlorhexidine and incubated at 25^{0}C.

RESULTS: Out of 150 clinically suspected cases,skin scrappings were collected from 63 patients and nail clippings 87.Among these KOH positivity was seen in 65 samples but culture positivity was 73 samples.The predominant pathogens isolated from skin and nail were dermatophytes 33(84%),Trichophyton mentagrophytes followed by Trichophyton rubrum and among candida species,candida tropicalis .Non dermatophytic moulds 9(26%)(Rhizopus sp.4,Fusarium sp.2,Aspergillus fumigates 2 and Aspergillus 1) were isolated from nail clippings.

KEYWORDS

Candida, Dermatophytes, DTM, NDM.

1.INTRODUCTION

Diabetes mellitus is a chronic metabolic dysregulation of glucose.It is a world wide problem of increasing importance. In Asia where two thirds of the world's diabetic live ,the type 2 DM accounts for 90% of the cases.[1].Cutaneous infection accounts for 20-50% of the skin manifestation among diabetic patients due to poor glycaemic control[1,2,3,4].More often these cutaneous signs heightens the suspicion of a physician regarding the diagnosis of the disease.

The fungal infections is the commonest cutaneous infection in diabetic patients.Moreover the prevalence of superficial mycoses namely onychomycosis and tinea pedis is 75% in diabetic patients especially in type 2.[5,6,7].The relative occurrence of the etiological agents of these superficial mycotic infections varies among countries[8]. Hence this study was conducted to know the prevalence and the causative agents in our region.

Diabetes is often associated with various serious complications , in particular diabetic foot ulcer. This causes considerable morbidity, disability and also leading cause for foot amputations [9]and hospitalisation. [10,11,12]

Onychomycosis and tinea pedis are the main predisposing factors for the development of these dreadful complications since it disrupts the skin integrity and allows the entry of secondary pathogens [bacteria]. [1,10]. Hence early detection of superficial mycoses especially tinea pedis and onychomycosis of toe nails which are not commonly noticed by the patient and timely adequate dosage of antifungal agents with good glycaemic control can save the limb.

Skin and nail infections are more common in diabetes [13].The commonest causative agent are the dermatophytes[57%].Candida species constitute 28% of cutaneous infection [5] and its incidence is high in diabetes because of the decrease in the β globulin ,an anti candidal factor [4].

Several Candida species are involved in human infections .Among these, Candida albicans is the most common species. However during the past decade, there is an emergence of non albicans Candida species especially among the diabetic patients due to immunosuppression [14].Apart from these agents ,uncommon non dermatophyte moulds can also cause2-7% of superficial fungal infection in diabetic patients these includes Aspergillus spp,Fusarium spp,Scytalidium spp, Acremonium spp and Rhizopus spp [15].

Thus diabetic patients due to their immunosuppressive states are prone for frequent fungal infections of the skin and nails . Hence their early diagnosis ,isolation of the causative agents [16] can prevent major complications.

2.AIMS & OBJECTIVES

1. To isolate and identify the casautive organisms of superficial mycoses in diabetic patients.
2. To study the prevalence of superficial mycoses in diabetes mellitus.

3.MATERIALS AND METHODS

The study was conducted after obtaining approval from the institutional ethical committee.

3.1.Study period- One year (October 2011-September 2012)
3.1.1Study setting-Institute of Microbiology,Madras medical college,Chennai
3.1.2Study design-Cross sectional
3.1.3.Sample Size-150

3.2.Inclusion crtiteria: All diabetic patient (type I & II),both male and female patients attending diabetology or dermatology OPD and those admitted with fungal infections of skin,hair & nail.

3.3.Exclusion criteria:

1. Patients on immunosuppressant drugs, systemic antibiotic therapy, oral contraceptive pills and long term steroids.
2. Autoimmune disorders.

4.Methodology

Samples- Skin scrapping,Nail clipping and plucked hair

All samples collected are microscopically examined with 10-40% KOH wet mount and inoculated in duplicate onto SDA with gentamicin,SDA with gentamicin and chlorhexidine .One set incubated at 37^0 C and second set at 25^0 C.One set of Dermatophyte test medium [DTM]containing chloramphenicol /gentamicin and cyclohexidine were also inoculated and incubated at 25^{0}C.

All the SDA tubes were read for week following incubation and twice

weekly thereafter for 4-6weeks.[17]DTM tubes are read for growth and colour change in the medium daily for 7-10days[17,18].Tubes showing no growth after the specified period were discarded.

Any visible growth on either of the slants was examined for colony texture,surface pigmentation,reverse pigmentation,presence of diffusible pigment and rate of growth.The isolates were identified using LPCB mounts and for dermatophytes urea hydrolysis test and invitro hair perforation tests were done for speciation.Creamy colonies are identified using Gram's staining,Germ tube test,Chrom agar and sugar fermentation tests.The data are statistically analysed.

5.RESULTS:

The 150 study population consisted of patients in the age group 26-85 years with 78 males(52%) and 72 (48%)females.There was preponderance of cases in the 41-50 years.The DM type II 132(88%) were more affected than DM type I 18(12%) patients. The samples collected were skin scrapings 63(42%)and nail clippings 87(58%) (Table-1). The predominant skin lesion was Tinea corporis 40[63%] followed by Tinea cruris 14(22%).In the nail lesions the predominant was DLSO 58[66%] followed by WSO 21(24%).

Table-1:Samples collected from study population(n=150)

Specimen	No of samples collected	Percentage (%)
Skin scrappings	63	42
Nail clippings	87	87
TOTAL	**150**	**100**

Out of the 150 clinically diagnosed cases,direct KOH mount positivity was seen in 35 skin scrapings and 30 nail clippings and the total KOH positivity was 65(43%)(Table-2).The culture positivity was seen in 39 skin scrapings and 34 nail clippings and the total culture positivity was73(48%).(Table-3).

Table-2 :Evaluation of KOH mount (n=150)

Specimen	KOH Positive	KOH Negative	TOTAL
Skin scrapings	35	28	63
Nail clippings	30	57	87
TOTAL	**65 (43%)**	**85(56%)**	**150**

Table-3: Evaluation of culture(n=150)

Specimen	Culture positive	Culture negative	TOTAL
Skin	39	24	63
Nail	34	53	87
TOTAL	**73(48%)**	**77(51%)**	**150**

In skin scrapings, dermatophyte 33 (84%) was the major pathogen isolated followed by Candida species 6(15%). Among the 33 dermatophytes, Trichophyton mentagrophytes 21(63%) was the commonest followed by Trichophyton rubrum 9(27%), Trichophyton verrucossum 2(6%) and Trichphyton tonsurans1(3%). Among the 6 Candida species, the major isolate was Candida tropicals 4 followed by Candida parapsilosis and Candida guillermondii each 1 isolate. (Table-4&5).

Table-4: Isolates in study population (n=150)

Isolates	Skin	Nail	TOTAL (%)
Dermatophyte	33	14	47(64%)
Candida	6	11	17(23%)
Non Dermatophytes	0	9	9(12%)
TOTAL	**39**	**34**	**73**

In nail clippings, dermatophyte 14(41%) was the major pathogen isolated followed by Candida species 11(32%) and non dermatophyte moulds (NDM) 9(26%). Among the 14 dermatophytes,Trichophyton mentagrophytes 12(85%) was the commonest followed by Trichophyton rubrum 2(14%). The 9 non dermatophyte moulds were 9(26%), namely Rhizopus species 4,Fusarium species 2, Aspergillus fumigates 2 and Aspergillus nidulans 1.Among the 11 Candida species, the predominant isolate was Candida tropicalis 7, Candida guillermondii 3 and Candida parapsilosis 1 isolate. (Table-4&5).

Table-5: Clinicomycological pattern (n=73)

Clinical condition	Dermatophyte	Candida	Non dermatophyte
Tinea faceei	1	0	0
Tineabarbae	1	0	0
Tinea corporis	21	0	0
Tinea manuum	1	1	0
Tinea cruris	7	3	0
Tinea pedis	2	0	0
Web space	0	2	0
Tinea unguim	14	11	9
TOTAL	**47(64%)**	**17(23%)**	**9(12%)**

6.DISCUSSION

A total of 150 diabetic patients was clinically diagnosed as case of superficial mycoses were studied during the one year period.The age group were 26-85,the mean age being 55.5years same as that studied by Ditte Maria et al(2006)[19]. There was preponderance of cases in the 41-50 years similar to Blanka Havlickova et al (2008) [20,21]. There were 78 males(52%) and 72 females(48%) similar to M.Situm et al (1998) and Kennedy et al (2007). [19,21,22,23,24].

The distribution of type of DM in the study group were typeI 12% and type II 88%, similar to Eckhard et al (2007).[12,19,22].Out of the 150 DM patients studied ,47 showed PPBS <160mg% and 103 >160mg%.The infection rate among the blood sugar controlled was 59% and uncontrolled 43%.

Thus Stastistical analysis based on Fischer exact test showed DM patients are susceptible to superficial mycoses irrespective of their age,gender,type of DM and blood sugar level.[5,19]

A total of 63 skin scrapings and 87 nail clippings(Table-1) were collected as shown by Phoebe Rich et al that onychomycosis was the most common infection among DM.[15,19,22,25].Among the skin lesions,tinea corporis was the predominant followed by tinea cruris. As stated by Verenkar MP et al(1991) that the high incidence in these sites was due to its symptomatic nature .Among the nail infection DLSO was predominant similar to Garg et al (2004).[21,26,27].

Out of 150 clinically diagnosed cases,direct microscopic examination (KOH) showed positivity in 35skin scrapings and in 30 nail clippings. Thus the total KOH positivity was 65(43%) which was similar to JC Mohanty et al (1999) 43%[28].(Table-2)

The casual agents were isolated by culture in 39 skin scrapings and in 34 nail clippings. Thus the total culture positivity was 73 (48%) which only slightly varied with Pankajalakshmi et al Chennai (1981), 44%; Sharma N L et al,Shimla (1987) 45% and Mallick AK et al.Rohtak (1996) 5 3%. (Table-3)

Thus a total of 35 (23%) samples were positive on direct microscopic examination and culture which was similar to Mallick AK et al (1996) 23% and slightly lower than Vasu BH et al, Warangal (1966) 26%.

In skin lesions, dermatophyte was the major pathogen isolated which accounted for 33(84%) out of culture positive cases followed by Candida species 6(15%) as shown by Lugo.Somolinos et al 15% of isolates in DM were candida.[19,29,30,31,32].Among the dermatophytes, Trichophyton mentagrophytes 21(63%) was the predominant followed by Trichophyton rubrum 9(27%) (Table-4&5) similar to M. Situm et al (1998) and Romano C et al (2001) [14,20] and the predominant candida species was Candida tropicalis 4(66%).

In nail infection,dermatophyte was the major pathogen which accounted for 14(41%) out of the culture positive cases followed by candida 11(32%) and non dermatophytic mould 9(26%).Among the dermatophytes isolated , Trichophyton mentagrophytes 12(85%) was the commonest followed by Trichophyton rubrum 2(14%) similar to Macura AB et al (2007)[14].The predominant candida species was candida tropicalis 7(63%) and among the non dermatophyte moulds (repeat isolates),the predominant was Rhizopus species 4(44%) followed by Aspergillus fumigates and Fusarium species each 2 isolates [23,33,34,35,36] (Table-4&5) as W g Cdr Grover S et al (2003) stated that NDM colonizes the damaged tissue and can cause infection in DM patients and moreover Mohammed Rahbar et al (2010) explained that the spores of NDM which are ubiquitous in the environment can transiently colonize healthy skin and causes debilitating diseases in immunocompromised patients.

7.CONCLUSION

Diabetes is increasing worldwide and the mortality due to diabetic foot syndrome is also on the rise. Superficial mycoses accounts the common cutaneous manifestation and especially onychomycosis which can act as a chronic reservoir. Due to associated complications

like neurovasculopathy ,obesity and hyperglycaemia ,the tissues are further damaged before the patient can recognise the lesion . The chronicity and tissue disruption can lead to secondary bacterial infection.

Clinical suspicion ,early laboratory examination to confirm diagnosis and appropriate treatment is very crucial in this group of patients. Diabetic patients are susceptible to superficial mycoses irrespective of their age, gender, type of DM and blood sugar level as proved with statistical analysis. Thus their immunosuppressive state is the main risk factor .

Direct microscopic examination and culture identification plays the major part in the management. Though Sabouraud dextrose agar with antimicrobials which needs incubation for 4-8weeks, is the commonly used media for isolation, Dermatophyte test medium which give presumptive identication within a week can be used instead in these group of patients .

Other agents like Candida and non dermatophyte moulds which were considered as contaminants or colonizer is also emerging as a pathogen especially in these group of patients. Hence repeated isolation will prove its pathogenicity.

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