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Original Research Article

Clinical study of coexistence of fungal infections in diabetic foot ulcers and its management

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ABSTRACT

Background: Presently 62 million Indians are diabetics and these numbers are on the rise. Amongst chronic complications of uncontrolled diabetes, foot ulcer is one. Diabetes is the leading cause of non-traumatic lower extremity amputation in developing countries with risk being 15 to 46 times higher. Infections are predominantly polymicrobial, predominantly Aerobic Gram-Positive cocci with higher incidence of anaerobic species and fungal infections. Treatment of fungal infections in diabetic foot ulcers might reduce the disability, morbidity and mortality in diabetic patients.
Methods: A total of 100 diabetic foot ulcer patients admitted or who visited to JSS Hospital over a period of 2 years, meeting the inclusion/exclusion criteria of the study, formed the study population. Detailed history, clinical evaluation & necessary investigations were done. Fungal isolation from the ulcer done by 10% KOH study, Gram stain, SDA culture and slide culture methods. Positive patients were treated with antifungal agents. Outcome of the disease studied based on ulcer progression, wound healing and tissue amputations.
Results: Polymicrobial flora in diabetic foot ulcers was seen (137 organisms in 100 subjects), predominantly Enterobacteriaceae and Pseudomonas. Prevalence of fungal infections in present study was 19%, of which Candida species was the commonest.
Conclusions: Present study signifies the need of mycological evaluation of nonhealing diabetic ulcer, with poor progression despite antibacterial therapy and foot care, introduction of antifungal treatment for proved fungal infections in diabetic foot ulcers, considering fungal infection as significant risk factor.

Keywords: Mycology, Polymicrobial, SDA (Saboraud's dextrose agar)

INTRODUCTION

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 the year 2030.[1] Overall incidence of diabetes in India is 1.1%. India today leads the world with its largest number of diabetic subjects in any given country. It is said that presently 62 million Indians are diabetics and these numbers are likely to increase to 89.4 million by the year 2025.This will be 1/6thof the world's total diabetics. Current prevalence rates are 12.1% in the urban Indian adult population.[2] There is evidence that the prevalence is increasing in rural population also. India is already the diabetes capital of the world.[3]

Out of the two types, the incidence of Type-2 DM is very high amongst Indians with 94-98% of Indian diabetics belonging to this group. The number of detected cases of DM reflects only the tip of the iceberg, because in India there is a larger number of undetected cases than of

detected cases.[4] The death in each year is due to its complications, which are common in age group of 40-60 years affecting both sexes equally. The complications are more prevalent among the lower socioeconomic status because of negligence, illiteracy and poverty etc.

Among the several chronic complications of uncontrolled diabetes, foot ulcer is one. Approximately 15% of individuals with DM develop a foot ulcer (great toe or MTP areas most common).[1,2] Diabetes is the leading cause of non-traumatic lower extremity amputation in developing countries. The risk of lower limb amputation is 15 to 46 times higher in diabetics than who are not.[5,6] It is mentioned by specialists that 20 out of 100 diabetic Indians undergo lower limb amputation. The reasons 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. Diabetic ulcers occur due to three factors:

- Trophic changes resulting from peripheral neuritis
- Atheroma of the arteries resulting in ischemia
- Excess of sugar in the tissues, which lowers resistance to infection, including fungi.[7]

METHODS

Source of data

All eligible cases attending Admitted to surgery wards in JSS Hospital Mysore for treatment of Diabetic Foot Ulcer during the period of study from December 2015 to September 2017. The data was collected from 100 subjects fulfilling the inclusion/exclusion criteria, admitted in JSS Hospital, using a proforma specially designed for the study. Descriptive type of the study design was used. Sample size was 100. Duration of study period was from December 2015 to September 2017.

Inclusion criteria

- Patients attending admitted in surgery wards in JSS Hospital for diabetic ulcer management
- Diabetic patients with blood glucose levels under control
- Non-healing ulcers of duration>2months on antibiotics and wound care.

Exclusion criteria

- Ulcers on foot other than diabetic like secondary to venous disorders, arterial diseases
- Diabetic ulcers with systemic infection
- Patient on anti-fungal treatment
- Patients allergic for anti-fungal
- Pregnant and lactating women.

Sample collection: Ulcer was cleaned with Povidone Iodine solution and Sterile Normal Saline, samples for the study were taken from the depth of the ulcers measuring around 0.5x0.5cms. Transport media: tissue samples were collected in plastic bottles (autoclavable) containing approx. 4-5ml of Normal Saline in it. Tissue samples from the ulcers under aseptic precautions, sealed and labelled and were taken to Microbiology Lab within an hour.

Processing of samples

Tissue blocks received were taken out from the sample collection bottles and were Triturated & then the tissue was ready for processing. Prepared tissue sample was then subjected to:

Preliminary examination

KOH, 10% Preparation

Tissue specimen is placed on a clean slide and 10% KOH is added, left in the incubator at 37°C for 2 hours. Cover slip is placed and examined under microscope. The alkali digests the keratin tissue and other tissue materials enabling the fungus elements to be seen clearly. Content: Potassium Hydroxide 10 gm dissolved in 100ml of Distilled Water. First, examination is done under the low power with reduced light and looked for fungal elements.

Gram stain

With the triturated tissue material, a smear is made on a clean slide, which is then dried, heat fixed and stained with Gram stain. Contents: Crystal Violet: Weight Crystal Violet (1.0gm); add in 5% Sodium bicarbonate 1.0ml solution and mix it. Make up the volume to 100ml with Distilled water.

Gram's Iodine

Weight Iodine crystals (2gm); add in freshly prepared Sodium Hydroxide solution (10ml); and make up the volume up to 100ml with Distilled Water. Gram stained smears are examined under oil immersion for the presence of Gram positive budding cells and pseudohyphae. This is particularly important when Candida is suspected.

Cultural procedures

Samples are inoculated onto 4 tubes containing Sabouraud's Dextrose agar with antibiotics-Chloramphenicol (2 of them with Actidione). One pair of culture tubes (SDA+AB with and without Actidione) is then incubated at Room Temperature (25-30°C) and the other pair at 37 °C respectively. Culture is examined twice in a week up to 4-6 weeks before declaring negative. Sensitivity for anti-fungal agents was done. Contents: Sabonrauds Dextrose Agar (SDA)- Weight Dextrose (40mg), Neopeptone (10mg), Agar (20mg) and make the volume up to 1000ml Distilled water. Adjusted

the final PH - 5.6. Chloramphenicol 5mg or Gentamycin 0.5mg antibiotics are used to inhibit the bacterium. Actidione (Cycloheximide) - 50mg are used to inhibit the growth of contaminant Saprophytic Fungi.

Lactophenol 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 grown. The filaments and spores are stained light blue. Contents: Phenol (liquefied) 20 ml; acts as disinfectant. Lactic acid 20 ml; preserves the morphology of fungi. Glycerin 40 ml; hygroscopic agent prevents drying. Cotton blue 0.05 gm; stains outer wall of fungus. Mix all the content and make up the volume to 20ml with Distilled water.

Slide culture

LPCB mounting sometimes disturbs fungal morphology during the process, hence Slide culture is done to confirm the INTACT morphology of the fungal isolate.

Slide culture technique

- Agar block taken to inoculate the colonies grown
- Slide culture dish with nutrient agar
- Inoculated block is placed in the dish
- Cover slip placed over it.

Intervention to subjects

Once the KOH study shows Positive, then Oral Anti-fungal agent was started. Oral Fluconazole 150mg weekly for about 6 weeks to 6 months depending on the progression of the ulcer. Fungal infection and type was confirmed with growth on SDA culture media. If SDA shows no growth after 1 month, then it is declared as negative. Type of fungal infection, its morphology was later confirmed with LPCB mount or Slide culture.

The other supportive wound care measures like Glycemic control with Insulin, Anti-biotic as per Pus Culture & Sensitivity, relieving of pressure over the affected part of the foot and the progression of the wound was studied. Dressing of the wound was done regularly. Any surgical intervention required during the course of the study, based on ulcer progression was done.

Follow up

Each ulcer was followed upto 6 months or till the ulcer healed, from the time of inclusion in the study and documented regarding the progression of ulcer based on ulcer grade, healing of ulcer based on requirement of any method of surgical intervention. Fungal positive patients who were started on Anti-fungal therapy were followed up every 15 days till the time of ulcer healing or 6 months. The other fungal negative ulcers were followed once a month.

Progression of ulcer in patients on anti-fungal therapy was documented based on ulcer grade, duration of ulcer healing and any surgical intervention required. Finally, whether amputation was required for any of the diabetic foot ulcer patients. Results obtained were analyzed and discussed. Conclusions were made based on the factors influencing the fungal positivity in Diabetic foot ulcers, the incidence of fungal infection co-existing in diabetic foot ulcer in our hospital, fungal and bacterial co-existence and the outcome of the ulcers based on ulcer grades.

RESULTS

An observational study consisting of 100 patients with diabetic foot ulcers is undertaken to study the incidence of fungal isolation and its correlation with various clinical features. In the present study the age variation was from 35 to 85 years with Mean±SD:58.21±11.96. Majority of in the age group of 51-60 years included 29 patients (29%).

Table 1: Grade of ulcer.

Grade of ulcer	No. of patients	Percentage
Grade II	39	39.0
Grade III	43	43.0
Grade IV	18	18.0
Total	100	100

Majority of subjects in the present study were Males (71%) as compared to females (29%). Majority of foot ulcers include in our study were secondary to some Trauma (56%). Duration of ulcer varied from 2 months to 8 months with Mean±SD:3.76±1.39. Majority of them were between 2-3 months (43%). Majority of foot ulcers in our study belonged to Wagner's Grade III ulcers (43%.). Majority of patients in our study had DM for more than 5 years (58%).

Table 2: Duration of diabetes.

Duration in years	Patients	%
<5	42	42.0
5-10	36	36.0
10-15	14	14.0
15-20	5	5.0
>20	3	3.0
Total	100	100.0

Table 3: Co-morbid conditions of patients.

Co-morbid	Total no. of patients (n=100)	%
HTN	38	38
CRF	5	5
IHD	14	14
TB	2	2
Hypothyroid	1	1

Most common co-morbid state present in our subjects was Hypertension (38%).

Microbiological observations

In 100 patients studied, 137 organisms (118 aerobic bacteria and 19 *fungi)* were isolated. Specimens from 8 (8%) ulcers were sterile and did not grow any bacteria or fungi. Among 92 specimen showing growth, only bacteria were isolated in 73 cases (79.35%), whereas mixed infection of bacteria and fungi was found in 13(14.13%) cases.

More than one bacterium was grown in 18 (19.56%) specimen. Polymicrobial flora was seen in 3 1 (33.7%) specimen, whereas Monomicrobial flora in 61 (66.3%) cases. Among the bacterial isolates, gram-negative comprised of 67% and gram-positive accounted for 32.7%. *Pseudoinonas aeruginosa* was the most common isolate, accounting for 27.88%; followed by *Staphylococcus aureus, Enterococcus spp. and Klebsiella spp.*, comprising 18.27%, 14.42% and 10.58% respectively (Table 4).

Table 4: Bacterial Isolates.

Bacterias	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
Acenitobacter	5	4.8
Citrobacter	5	4.8
>1 bacteria	18	17.3
No growth	14 (14%)	

Fungal isolates

Out of 100 subjects included in our study, 19(19%) of them had fungus grown in the tissue specimen. Out of 19, six (31.58%) of them were isolated growths (i.e. they were not associated with any bacteria) as proved by Pus c/s. The remaining 13 (68.42%) co-existed with at least one bacteria in the ulcer. Pseudomonas (53.85%) was the commonest bacterium co-existing with fungal infection. Second commonest was Staph. aureus accounting for 30.77% of mixed infection. The list of all fungus isolated and their characteristics:

1. Candida: (5 counts)

Two of them were (Germ Tube +ve), remaining 3 were Germ Tube - ve. 10% KOH Preparation: Yeast like cells seen Gram Stain: Gram +ve, spherical, budding yeast-like cells, approx 3-6 µm in size. SDA Culture: macro Cream, pasty colonies were seen. Micro: Gram Stain of these growth showed Gram +ve, spherical, budding yeast-like cells with Pseudohyphae.

2. Trichosporon: (1 count)

SDA Culture - Growth seen in 3-6 days. Macro: Initially creamy, later turned yellowish-grey and wrinkled. LPCB mount: Micro: Hyaline hyphae with oval arthrospores.

3. Aspergillus: (4 counts)

Two of them were Aspergillius niger, and one each of A. flavus and A. fumigatus. 10% KOH: Septate fungal filaments seen in all types.

Aspergillus Niger

SDA Culture-Growth was seen in 2-6 days. Macro: Black powdery colonies. LPCB mount - Micro: Vesicle spherical with biserrate sterigmata showing primary and secondary phialides/ sterigmata.

Aspergillus fumigates

SDA Culture: Growth occurred in 4-6 days. Macro: Bluish-green, Powdery colonies. LPCB mount - Micro: Inverted flask shaped vesicle with uniserrate sterigmata on upper ½ of the vesicle.

Aspergillus flavus

SDA culture: Growth seen in 2-5 days. Macro: Yellowish green, powdery colonies. LPCB mount - Micro: Rounded vesicle with biserrate sterigmata seen.

4. Fusarium: (3 counts)

SDA Culture: Growth seen in 3-6 days. Macro: Fluffy colonies, pink in colour. LPCD mount- Micro: Septate hyphae with phialides which produce single celled micro-conidia or large boat/sickle shaped macro-conidia with numerous septa.

5. Trichophytons (Dermatophyte): (3 counts)

One of them was Trichophyton rubrum, other two were not speciated. SDA Culture: Growth was seen in 2-3 weeks. Macro: White, downy, granular, velvety with regal folds were seen. Trichophyton rubrum on reverse, deep red colour was seen. LPCB mount - Micro; Septate hyphae with tear shaped micro-conidia seen along the sides with no macro-conidia. In T. rubrum, pencil shaped macro-conidia were seen.

6. Penicillium: (2 counts)

SDA Culture - Growth seen in 3-6 days. Macro: Bluish green, velvety to powdery colonies. LPCB mount - Micro: Septate hyphae with brush-like conidiophores

were seen.

7. Acremonium (Cefalosporium): (1 count)

SDA Culture - Growth seen in about 3-5 days. Macro: Initially Greyish, then later turned Reddish-orange colonies. LPCB mount - Micro: Septate hyphae with tube-like phialides.

Fungal isolates in our study

19 fungi were isolated, commonest being *candida* (5) accounting for 26.32%, followed secondly by *Aspergilus* (4) accounting for 21.05%. Morphologically fungal isolates were classified as Yeasts and Molds. Yeasts were 6 in number (31.58%) and Molds were 13 (68.42%). Among yeasts, *Candida* was commonest: 5 (83 .33%). Among Molds, highest incidence was *Aspergillus* species: 4 (30.77%). Other fungal elements isolated in our study are shown in the Figure below:

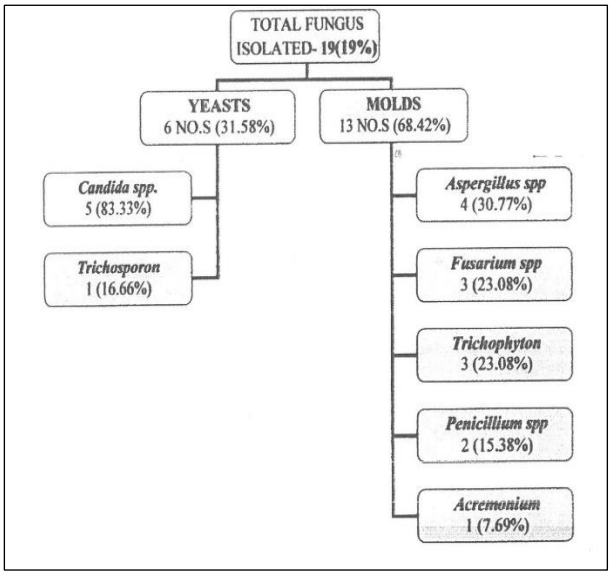

Figure 1: Fungal elements isolate.

Result analysis of data for factors influencing fungal infection

Table 5: Duration of ulcer (months).

Duration	Patients	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	003**
5-6	9	3	33.33	0.273
>6	3	0	0.00	-
Total	100	19	19.00	-

Fungal positivity in our study was commonly found in ulcers of duration between 4-5 months and is statistically significant and associated with fungal infection.

Table 6: Duration of DM (years).

Duratio	Patients	Positive fungal isolation	%	P value
<5	42	8	19.05	0.993
5-10	36	7	19.44	0.946
10-15	14	3	21.43	0.817
15-20	5	1	20.00	0.955
>20	3	0	0.00	-
Total	100	19	19.00	-

Table 7: Blood sugar levels.

Levels (mg/dl)	Patients	Positive fungal isolation	%	P value
<100	6	2	33.33	0.371
101-140	41	7	17.07	0.753
141-180	28	5	17.86	0.888
181-220	18	4	22.22	0.729
>220	7	1	14.29	0.751
Total	100	19	19.00	-

Duration of ulcer and blood sugar levels of diabetic patients do not show any correlation with fungal infection.

Table 8: Bacterial isolation.

Bacterial isolation	Patients	Positive fungal isolate	%	P value
Pseudomonas	29	7	24.14	0.481
Staph. aureus	19	4	21.05	0.819
Enterococcus	15	1	6.67	0.223
Citrobacter	5	-	-	-
Proteus	6	-	-	-
Klebsiella	11	1	9.09	0.402
E. Coli	7	-	-	-
Acetinoobacter	5	-	-	-
Enterobacter	7	-	-	-
No growth	14	6	42.86	0.023*
>1 bacteria	18	-	-	-
Total	100	19	19.0	0

There was a significant association between fungal infection and Ulcers having No growth on Pus C/S. Isolated fungal infection is significantly more common owing to long term antibiotic therapy inhibiting any bacterial growth in the ulcer.

Table 9. Intervention on follow up.

Intervention required	Patients	Positive fungal isola	%	P value
Dressing	28	6	21.43	0.743
Sec. suturing	25	4	16.00	0.702
Split skin graft	24	8	33.33	0.074±
Amputation	23	1	4.35	0.073±
Total	100	19	19.00	-

Also, shown in above Table, that fungal infection co-existing with bacteria is more common with Pseudomonas. Fungal positivity in our study was significantly associated with secondary suturing (most common) and disarticulation/amputation (least common). Inference: treating fungal infection in diabetic foot ulcers, significantly leads to limb salvage.

Statistical analysis

Descriptive statistical analysis has been carried out. Results on continuous measurements are presented on Mean±SD (Mm-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Chi-square/2z2, 2x3, 3x4 Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Single proportion Z-test has been used to find the significance of incidence of Fungal positive in relation various clinical characteristics.

Fisher Exact test

Let there exist two such variables x and y, with ^{m} and observed states, respectively. Now form an mXl2 matrix in which the entries Are present the number of observations in which x=1 and v=j. Calculate the row and column sums and respectively, and the total sum of the matrix. Then calculate the conditional probability of getting the actual matrix given the particular row and column sums, given by which is a multivariate generalization of the hypergeometric probability function.

Z-test for a proportion (Binomial distribution)

Objective of the test is to investigate the significance of the difference between assumed proportion and the P^{0} and the observed proportion P.

Significant figures

±Suggestive significance (P value: 0.05<P<0.10). *Moderately significant (P value:0.01<P ≤0.05). **Strongly significant (P value: P≤0.01).

The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

DISCUSSION

In 100 cases of diabetic foot ulcers taken, 71% of them were males and 29% females. The age ranged between 35 and 85 years with a Mean±SD:58.21±11.96.

Subjects included in showed that foot complications are common in the elderly patents. Most of the ulcers included in present study belonged to Wagner's Grade II (39%) and Grade III (43%) and were secondary to some Trauma. However, other studies like Bansal et a1 (or III- 35.9%, or IV- 44.66%) and Emilija et al (III36.4%, IV- 3 1.8%) have encountered with higher number of Grade III and IV ulcers.[8,9]

The duration of ulcers in the present study ranged from 2 months to 8 months with Mea±SD:3.76±1.39 months. Majority belonged to 2-3 months (43%). Similarly, Emilija et al study also had mean duration of ulcer to be 3-4 months.[9]

Table 10: Age and Sex Distribution.

	Bansal et al[8]	Raja et al[10]	Present study
Male:Female (%)	79:21	61:39	71:29
Mean age±SD (years)	57.04±11.63	57.6	58.21±11.96
Age group	51-70	51-70	51-60

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Table 11: Organisms per case comparison with other studies.

	Vishwanathan et al[11]	Raja et al[10]	Bansal et al[8]	Present study
Organisms per case	1.21	1.47	1.52	1.37

The duration of ulcers in the present study ranged from 2 months to 8 months with Mean±SD:3.76±1.39 months. Majority belonged to 2-3 months (43%). Similarly, Emilija et al study also had mean duration of ulcer to be 3-4 months.[9] Polymicrobial flora was seen in 31 (33.7%)s, monomicrobial flora in 61 (66.3%) cases. Similar results of Polymicrobial infection has been observed in various studies.[8-13]

Table 12: Bacterial isolates-comparison.

	Bansal et al[8]	Present study (%)
Polymicrobial	29.32	33.7
Gram –ve organisms	76	67.3
Gram +ve organisms	24	32.7
Commonest Pseudomonas	21.67	27.88
2nd common Staph. aureus	18.88	18.27

Gram negative organisms were more predominant than Gram positive organisms. Similar results were seen in study by Bansal el al as shown in Table 12.[8]

Table 13: Fungal isolates compared with the other studies.

	Bansal et al[8]	Emilija et al[8]	Present study
Total subjects	103	509	100
Fungal isolates	9%	4.5%	19%
Most common	*Candida* (29%)	*Candida*	*Candida* (26.32%)
2nd common	*Aspergillus* (21%)	-	*Aspergillus* (21.05%)

Out of 100 subjects included in our study, 19% of them had positive fungal growth. Similar results were seen in other studies like in Bansal et al with 9% fungal growth, candida being commonest. Emilija et al grew only Candida species.[8,9]

Out of 19 fungi grown, six (31.58%) of them were isolated growths. The remaining 13 (68.42%) co-existed with at least one bacteria in the ulcer. This was comparable with study by Emilijaet al.[9]

Table 14: Bacterial and fungal co-existence.

	Emilija et al[9]	Present study
Pure fungal infections	31.8%	6 (31.58%)
Mixed fungal infection	68.2%	13 (68.42%)
Commonest bacteria	*Enterobacteriaceae* and *Pseudomonas*	*Enterobacteriacea*, *Pseudomonas* and *Staph. aureus*

Morphologically fungal isolates obtained in our study were classified as Yeasts and Molds. Yeasts were 6 in number (31.58%) and Molds were 13 (68.42%). Among yeasts, *Candida* was the commonest- 5 no. s (83.33%) and among Molds, highest incidence was *Aspergillus*- 4 no.s (30.77%). Results obtained in other studies are shown in Table 15.

Table 15: Morphological classification-comparison.

	Bansal et al	Seema Nair et al	Present study
Yeasts	50	66	31.58
Highest *Candida*	92	82	83.33
Molds	50	34	68.42
Highest *Aspergillus*	42	65	30.77

Bansal et al study had equal number of Yeasts and Molds isolated (50% each), but in Seema flair et al study, yeasts (66%) were more than molds (34%) which is opposite to our study.[8,13] Among yeasts, *Candida* was the commonest and *Aspergillus* among molds showed highest incidence in all.

It was seen that ulcers were more common in Males, age group of 51-60 years, and in Wagner's Grade II and III ulcers, occurring secondary to trauma. Present study shows that ulcers of duration between 4 -5 months (P value: 0.003) showed a significant association with fungal positivity. Also, Ulcers with no growth in Pus C/S (i.e. sterile) (P value- 0.023*) had a significant association with fungal infection.

On follow-up, it was noticed that Ulcers with fungal infection on anti-fungal therapy had a good prognosis & course of ulcer progression, with significantly less amount of tissue loss (P value- 0.004**) and requiring only conservative management (P value- 0.074+) for healing. Similarly, studies by Emilija et al and Seemanair et al have shown poor treatment outcome without administration of specific therapy in fungal infected ulcers, thus justifying the introduction of systemic antifungal therapy in patients with verified fungal ulcer infections.[9,13]

CONCLUSION

Present study shows Polymicrobial nature of Diabetic foot ulcers, predominantly with Enterobacteriaceae and Pseudomonas, with fungal infection accounting for 19% of cases. It was more common in long-standing ulcers (4-5 months). The incidence of Pure fungal infection was more (42.86%) compared to mixed infection. Treating these proved cases with anti-fungal agents, showed improvement in disease status and a significant reduction in tissue loss, with faster wound healing process.

The present study signifies the need of a mycological evaluation of a non-healing diabetic ulcer of a longer duration, with poor progression despite antibacterial therapy and foot care and introduction of antifungal treatment for proved fungal infections in Diabetic foot ulcers. We believe that our results will create awareness among clinicians, of the need to treat fungal and mixed fungal-bacterial infections of diabetic foot ulcers, as well as encourage further research into these infections.

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