

## Effectiveness of microscopy (using 10-20% KOH with or without lacto-phenol cotton blue) in identifying fungal elements in diabetic foot ulcer

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### Abstract:

**Objective:** Chronic, uncontrolled diabetes mellitus is associated with dysfunctions of the immune and healing systems. Consequently, repeated infections by bacteria and/or fungi and the sluggish healing process, pose a constant threat to the integrity of tissues, particularly the frequently injuring feet, leading to grave complications. This study assesses the identification of fungal elements in scrapings from diabetic foot ulcer through direct microscopy using 10% potassium hydroxide and lacto-phenol cotton blue.

**Material and Methods:** In this study, we included 298 diabetic patients with foot ulcers from June 2016 to June 2019. We recorded their demographic features, blood glucose at the time of clinical examination, and the grades of the ulcers according to Wagner ulcer grading scale. The samples from scrapings were taken with sterilized instruments from the edges of the ulcers and handled as per recommended guidelines.

**Results:** Fungal positivity was more in men (56.7%) than women (43.3%). Overall mean ( $\pm$ SD) age was 50.0 ( $\pm$  8.1). On fungal culture, total fungus positive cases were 30.9%. On simple microscopy, using KOH/LPCB, dermatophytes were identified in 95% ( $p < 0.05$ ) of the true positive cases.

**Conclusion:** The microscopy of skin scrapings from diabetic foot ulcer, using 10-20% potassium hydroxide and lacto-phenol cotton blue, is a simple and effective out-patient technique with an acceptable yield in terms of identifying fungal elements. Other similar studies have shown corresponding conclusions.

**Keywords:** Diabetic foot ulcer, bedside fungal testing, potassium hydroxide (KOH) wet mount and Lactophenol Cotton Blue (LPCB)

### Introduction:

Diabetes mellitus is the commonest endocrine disorder Duff et al.<sup>4</sup>, characterized by hyperglycemia as described by Eba et al.<sup>5</sup> Meo et al.<sup>13</sup> mentioned the population of diabetics is expected to exceed 640 million by the year 2040 Hussain and Ali<sup>8</sup> in his study mentioned that Pakistan, ranked 7<sup>th</sup> by World Health Organization on the list of diabetics inhabiting countries in 2016, is estimated to reach 4<sup>th</sup> position by the year 2030.

The chronic metabolic derangements associated with diabetes mellitus produce complications

which may be microvascular such as neuropathy, nephropathy and retinopathy and/or macrovascular such as coronary artery disease, stroke and peripheral artery disease as mentioned by Papatheodorou K. et al.<sup>15</sup> Eming et al.<sup>6</sup> in his study mentioned that immune mechanisms as well as wound healing processes are also compromised in chronic diabetes mellitus Mucocutaneous manifestations are frequent in DM and include both infective and non-infective conditions as described by Duff et al.<sup>4</sup> Mishra et al.,<sup>14</sup> and van Netten et al.<sup>18</sup> describes in their study that in diabetes, the presence microvascular, immune and

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Table 1: Socio-demographic characteristics of studied subjects (N=298)

Characteristics		N	%
Age Group	35 - 50 Years	183	61.4
	51 - 60 Years	80	26.8
	61 - 70 Years	35	11.7
	Mean $\pm$ SD (overall)	<b>50.0<math>\pm</math>8.1</b>	
	Mean $\pm$ SD (Male)	<b>51.48<math>\pm</math>9.0</b>	
Gender	Male	169	56.7
	Female	129	43.3

Table 2: Duration of diabetes mellitus and diabetic foot ulcer (N=298)

Duration		N	%
Diabetes Mellitus (years)	1 - 5	153	51.3
	6 - 10	98	32.9
	>10	47	15.8
	Mean $\pm$ SD	6.7 $\pm$ 4.6	
Diabetes Foot Ulcer (Months)	1 - 5	206	69.1
	6 - 10	44	14.8
	>10	48	16.1

Table 3: Association between dermatophyte positivity and fungus identification techniques used (n=49)

Parameters		Dermatophytes positivity				p-value
		Yes		No		
		N	%	N	%	
On Microscopy	Positive	47	95.9	40	16.1	<0.01*
	Negative	2	4.1	209	83.9	
On Culture	Positive	49	100.0	43	17.3	<0.01*
	Negative	0	0.0	206	82.7	

\* $p < 0.05$  was considered significant while  $p \geq 0.05$  was considered non-significant using Pearson Chi Square test

healing compromises as well as repetitive injury to feet, there is high risk of development of diabetic foot ulcers.

The immune dysfunction predisposes the diabetic foot ulcer to infection by bacteria or fungi as described by Akkus et al.<sup>2</sup> Giurato et al.,<sup>7</sup> in their study mentioned that foot infection particularly that involves bone requires major amputation in diabetics patient.

Diabetes is considered as one of the leading causes of lower limb amputations globally par-

ticularly when the progress of infection is not halted as described by Lazzarini et al.<sup>11</sup>

Bacteria are usually considered responsible for the infection of diabetic foot ulcer and treated accordingly. However, in a significant number of cases, the problem does not respond to antibacterial therapy. This is when search for another cause, such as fungi, is needed. In this study, we tried to assess the effectiveness of screening microscopy using potassium hydroxide (KOH) wet mount and Lactophenol Cotton Blue (LPCB) in identifying fungal elements in skin and tissue scrapping from diabetic foot ulcer.

### Material and Methods:

This study included 298 known diabetic patients, 169 males and 129 females, with foot ulcers, reporting in diabetic clinic of Jinnah Postgraduate Medical Center, Karachi. After taking written informed consent, demographic data of each patient was recorded on a printed questionnaire at the outset of evaluation. Information related to type and duration of diabetes, daily feet washing frequency as well as related to neuropathy and peripheral vascular system diseases were recorded. Blood glucose levels were checked. Diabetic foot ulcers were assessed according to Wagner-Meggitt ulcer classification scale which classifies diabetic foot ulcer into six grades as described by Jain and Joshi,<sup>10</sup>

Materials were collected according to the recommended guidelines, from the suspicious lesions in and adjacent to the ulcer, such as, intense squamous hyperkeratotic, or macerated area. Materials were then safely secured in sterile filter paper. The skin scrapping were examined microscopically under 40X magnification, using 10% potassium hydroxide (KOH) and Lactophenol cotton blue (LPCB), to identify fungal components such as hyphae and/or conidia. Specimens were labelled as "fungus positive" when these fungal components were identified. In case of absence of fungal components, this procedure was repeated twice at the same area before labelling the specimen as "fungus negative".

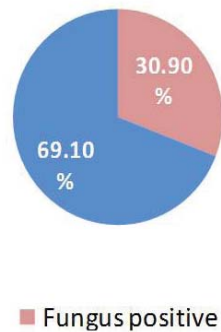


Figure 1: Prevalence of fungus in diabetic foot ulcer (N=298)

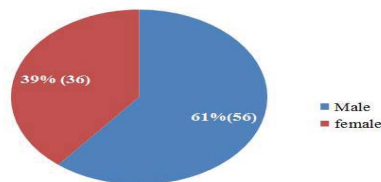


Figure 2: Frequency of fungus positivity in gender groups (n=92)

Statistical analysis of data was performed by using Statistical Package for Social Sciences program software version 23.0. Descriptive assessments and frequency analysis were performed.  $P < 0.05$  was accepted as statistically significant while  $P \geq 0.5$  was considered insignificant

#### Discussion:

The mean age of diabetics in our study was  $50 \pm 8.1$  years as shown in table-1. The mean age of diabetics reporting for diabetes related foot complications in other studies in Pakistan is similar to our findings. Maryam et al.<sup>12</sup> has mentioned the mean age of  $47.34 \pm 6.11$  years while Iqbal et al.<sup>9</sup> reported the mean age of  $54.50 \pm 10.89$  in their study.

We observed that adult diabetics with active age group were more affected with fungal infection of their foot ulcers. This is in concordance with the findings of Akkus G. et al.<sup>2</sup>

The prevalence of males with fungal foot infec-

tion was higher, more likely because they were more involved in outdoor activities and therefore more exposed to trauma and unhygienic conditions as well as they were less attentive about their foot hygiene. Our study showed male dominance similar gender predominance is mentioned by Mishra and Pratap<sup>14</sup> and Akkus et al.<sup>2</sup>

In our study, longer duration of diabetes was associated with higher frequency of fungal foot infection. This finding is consistent with the observation of Shahzad M. et al.<sup>17</sup> However, we observed no significant relationship between the frequency of fungal positivity and blood glucose levels at the time of sample taking. Romano et al. in his study in 2001 has similar observation which supports our findings. Akkus et al.<sup>2</sup> are of the opinion that hyperglycemia is associated with higher frequency of fungus positivity.

This research showed 92 out of the total 298 subjects to be positive for different types of fungi with the prevalence of fungi in diabetic foot ulcer calculated to be 30.9% as shown in figure-1. This is in conformity with study by Chellan et al.<sup>3</sup> who noted a prevalence of 27.2%.

In this study, it was observed that very few participants had known about fungal pathogens and their role in causing/sustaining foot ulcer. None of them were advised test for identifying fungal elements and none were prescribed any antifungal treatment on clinical suspicion. Moreover, simple fungal tests such as microscopy of ulcer scrapping were not included in any laboratory work up and hence not performed on any of these patients. This gap in the management of diabetic foot ulcer needs to be assessed and dealt with. We tried to evaluate this lacuna in the management of DFU and found out that the association between simple microscopy using 10% KOH (with or without LPCB) and fungal positivity was observed to be significant ( $p < 0.01$ ). A study by Ahmad et al.<sup>1</sup> has also observed KOH microscopy to be a simple yet effective out-patient technique in identifying fungi in onychomycosis.

Simple microscopy using 10% KOH and LPCB is an easy to perform bedside test to identify fungal elements especially dermatophytes in skin scrapping and is an integral part of every study on fungus. Dr. Ponka<sup>16</sup> also has regarded it as an important examination-based diagnostic procedure.

#### Conclusion:

We conclude that the concerned health professionals to include simple microscopy as a routine test for identification of fungi in diabetic foot lesions, particularly in case of non-healing or refractory lesions. This will not only help in selection of fungal positive patients for further study but also in starting empirical anti-fungal treatment long before the result of fungal culture.

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#### Role and contribution of authors:

Dr. Muhammad Khalid, collected the data, references and did the initial write up.

Dr. Muhammad Sabir, collected the data and helped in introduction writing.

Dr. Shahida Bashir, collected the references and helped in discussion writing.

Dr. Raziuddin Ahmed, collected the data, references and helped in introduction writing.

Dr. Fakhruddin Indhar, critically review the article and made final changes

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