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Research Article

Diabetes Mellitus and Fungal Keratitis: Comparative Aetiological and Diagnostic Profile in a Tertiary Care Ophthalmic Hospital

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ABSTRACT

Background: Fungal keratitis is a major cause of infectious keratitis and corneal blindness in tropical regions, with fungi responsible for 30-40% of cases. Diabetes mellitus increases susceptibility due to impaired epithelial healing, neurotrophic keratopathy, and immune dysfunction. Comparative data on etiological profiles, diagnostic yields, and fungal isolate distribution between diabetic and non-diabetic patients with fungal keratitis remain limited. This study aimed to compare the etiological and diagnostic profiles of fungal keratitis in patients with and without diabetes mellitus at a tertiary care ophthalmic hospital in Hyderabad, India.

Materials &Methods: This prospective observational study enrolled 100 consecutive patients with clinical features suggestive of infectious keratitis: 50 with diabetes mellitus (past 5 years) and 50 non-diabetic controls. Exclusion criteria included bacterial, viral, or parasitic keratitis and prior antifungal therapy. Corneal scrapings were collected from the ulcer base and advancing edge under topical anesthesia. Samples underwent direct microscopy (10% KOH mount), Gram staining, and culture on blood agar, chocolate agar, and Sabouraud dextrose agar (SDA) (25°C, up to 4 weeks). Fungal isolates were identified by colony morphology and lactophenol cotton blue (LPCB) mounts. Data were analyzed using descriptive statistics and chi-square test ($p < 0.05$ significant). Ethical clearance and informed consent were obtained.

Results: Diabetic patients were significantly older (74% >50 years; $p = 0.000005$) than non-diabetics (majority 30–49 years), with male predominance in both groups (64% vs. 70%). Trauma was the leading risk factor overall (32-40%), alongside foreign body exposure. Agricultural workers predominated (44% diabetics, 38% non-diabetics). Fungal culture positivity was 54% in diabetics and 50% in non-diabetics (overall 52%). KOH mount positivity was higher in diabetics (50%) than non-diabetics (34%). Fusarium spp. was the most common isolate in both groups (28% diabetics, 24% non-diabetics), followed by Aspergillus flavus (8% in both), Aspergillus niger (4–8%), Curvularia spp. (4-6%), Aspergillus fumigatus (2%), and unidentified isolates (2–8%). In diabetics, KOH-positive cases showed 23/25 culture-positive results; in non-diabetics, all 17 KOH-positive cases were culture-positive.

Conclusions: Fungal keratitis in diabetic patients showed higher KOH positivity and occurred in older individuals compared to non-diabetics, with Fusarium spp. predominant in both groups, consistent with tropical epidemiology. Diabetes influences diagnostic yield and demographic presentation but not markedly the fungal spectrum. Regional surveillance and prompt microscopy remain essential for diagnosis, while localized data guide empirical therapy amid rising diabetes prevalence. Further molecular studies are recommended for precise species identification and targeted management.

Keywords: *Fungal Keratitis, Diabetic Keratitis, Non-diabetic Keratitis.*

INTRODUCTION

Infectious keratitis, an inflammatory condition of the cornea, represents a significant ophthalmological challenge, often leading to visual impairment or blindness if not promptly and effectively managed. The etiological and epidemiological patterns of infectious keratitis exhibit considerable regional variation, influenced by climatic, geographical, and socioeconomic factors (1). Infective keratitis is a major cause of corneal blindness in developing countries, with fungi accounting for 30–40% of cases in tropical regions. Diabetes mellitus exacerbates susceptibility through delayed epithelial healing, neurotrophic keratopathy, and immune dysfunction(2). Diabetes Mellitus, a prevalent systemic metabolic disorder, is increasingly recognized as a significant predisposing factor for various infections, including those affecting the cornea (3). Given this predisposition, a deeper understanding of the specific causative agents and diagnostic considerations for fungal keratitis in diabetic patients is critical for optimizing treatment strategies and improving visual outcomes (4). This study aims to delineate the comparative etiological and diagnostic profiles of fungal keratitis in patients with and without Diabetes Mellitus presenting to a tertiary care ophthalmic hospital, thereby contributing to evidence-based clinical protocols. Specifically, it seeks to identify differences in the spectrum of fungal isolates, analyze the clinical characteristics, and evaluate the efficacy of current diagnostic approaches in both patient cohorts, ultimately informing targeted therapeutic interventions (5,6). Despite this, comparative data on fungal aetiology in diabetic versus non-diabetic patients remain limited. This study aimed to delineate differences in fungal culture positivity, direct microscopy yield, and isolate distribution. Furthermore, it investigated the impact of diabetes on clinical presentation and treatment outcomes in individuals with fungal keratitis.

MATERIALS AND METHODS

Present prospective observational study was conducted at the Department of Microbiology, Sarojini Devi Eye Hospital, Hyderabad, India. It enrolled 100 consecutive patients 50 with diabetes mellitus from past 5years and 50 non-diabetic patients presenting with clinical features suggestive of infectious keratitis. Exclusion criteria included cases of bacterial, viral, or parasitic keratitis, as well as patients who had received prior antifungal therapy.

Sample Collection: Corneal scrapings from the ulcer base and advancing edge using a sterile blade under topical anesthesia with the help of ophthalmologist under operating microscope.

Laboratory Procedures followed-

Direct microscopy: 10% KOH mount examined for fungal elements.

Culture:Corneal scrapings were inoculated onto solid media, including blood agar, chocolate agar, and Sabouraud dextrose agar (SDA), using C-shaped streaks. Material from subsequent scrapings was smeared onto glass slides for 10% KOH wet mount and Gram staining. Blood agar and chocolate agar plates were incubated aerobically at 37°C for 24-48 hours. Sabouraud dextrose agar plates were incubated at 25°C, monitored daily, and discarded after 4 weeks in the absence of growth. If there is a growth fungal isolates were identified by colony morphology and lactophenol cotton blue mount preparation (LPCB)(7,8).

Statistical Analysis: Descriptive statistics (percentages, tables, charts). Chi-square test for comparison ($p<0.05$ significant). Ethical clearance obtained from Institutional ethical committee and Patient consent was obtained from all participants prior to sample collection.

RESULTS

Figure 1: Infective Keratitis of Right eye

Figure 2: Specimen collection procedure

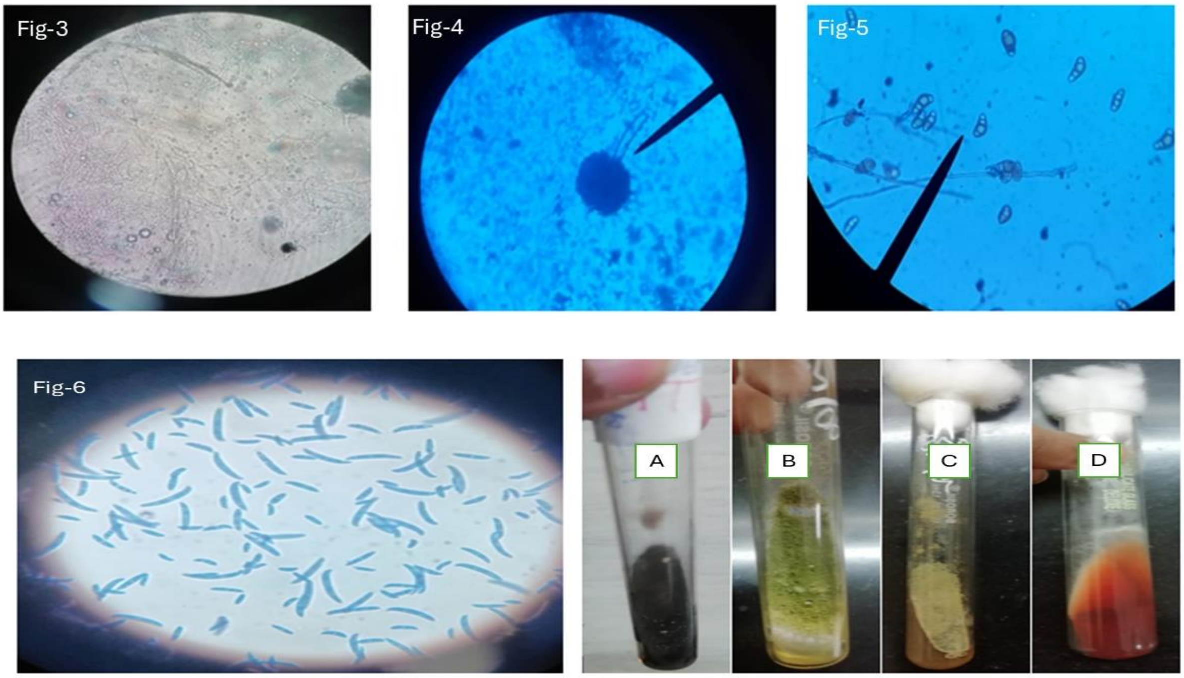

Fig3: KOH positive of corneal scrapping; **Fig 4**: LPCB of Aspergillus niger; **Fig 5**: LPCB of curvularia; **Fig 6**: LPCB of Fusarium; **A:** SDA slant of Curvularia;**B**: SDA slant of Aspergillus flavus;**C**: SDA slant of Aspergillus fumigatus;**D**: SDA slant of fusarium species.

Table 1 - Age distribution of patients:

Age in years	Diabetes	Percent	Non-Diabetic	Percent
20-29	0	0.00%	3	6.00%
30-39	2	4.00%	19	38.00%
40-49	11	22.00%	15	30.00%
50-59	19	38.00%	9	18.00%
>60	18	36.00%	4	8.00%
Total	50	100.00%	50	100.00%

Majority (74%) of the study population in the diabetic group belonged to the age group of > 50 years. 50-59 years age group constituted 38% and >60 years to 36% of the study population. 22% belonged to 40-49 years age group and 4% belonged to 30-39 years.Majority of the study population in the non-diabetic age group belonged to the age group of 30-49 years. 30-39 years age group constituted 38% and 40-49 years to 30% of the study population. 18% belonged to 50-59 years age group and 8% belonged to >60 years. 3% belonged to 20-29 years.The age difference between the two groups is statistically significant with P value of 0.000005

Chart 1 - showing age distribution of the study population

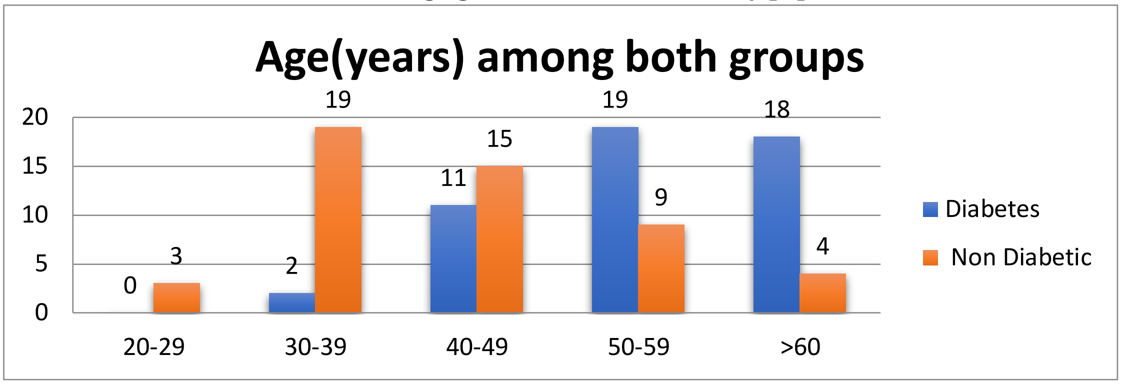

Table 2 -Gender of the study population

Gender	Diabetes	Percent	Non-Diabetic	Percent
Female	18	36.00%	15	30%
Males	32	64.00%	35	70%
Total	50	100.00%	50	100%

Among both the groups, male population outnumbered the females, indiabetic group, 64% were males and 36% were females and innon-diabetic group, 70% were males and 30% were females.

Chart 2: showing Gender of the study population:

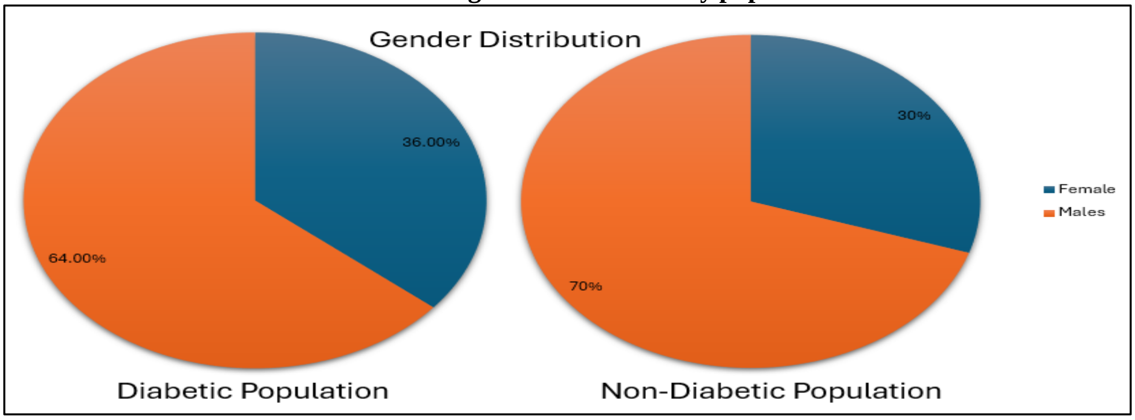

Table 3: Risk factors among the study population

RISK FACTOR	Diabetes	Percentage	Non-Diabetic	Percentage
Foreign Body	16	32.00%	17	34%
Post Surgery	8	16.00%	8	16%
Trauma	16	32.00%	20	40%
Unknown	10	20.00%	5	10%
Total	50	100.00%	50	100%

Among the total study population trauma was the most common risk factor present. Among diabetes, trauma and foreign bodies were the inciting factors among 32% each, followed by unknown factors (20%) and history of surgery (16%).Among non-diabetics, trauma was the inciting factor in 40%, followed by foreign body in 34%, past surgery in 16% and unknown factors in 5%.

Chart 3: Risk factors among study population

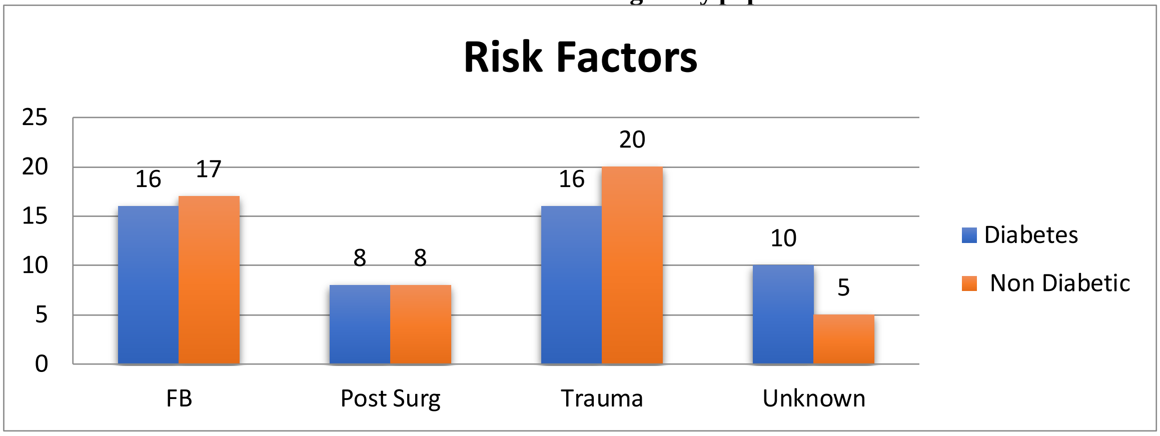

Table 4: Occupation of the study population:

OCCUPATION	Diabetes	Percent	Non-Diabetic	Percentage
Agricultural worker	22	44.00%	19	38%
Business	2	4.00%	5	10%
Household	3	6.00%	9	18%
Laborer	12	24.00%	10	20%
Professional	1	2.00%	0	0
Unemployed	10	20.00%	7	14%
Total	50	100.00%	50	100%

Among 2 groups most of them were agricultural workers.Diabetic group, 44% were agricultural workers, 24% were daily wage earners, 20% were unemployed, 6% were household workers, 4% were businessmen and 2% were professionals. Among the non-diabetic group, 38% were agricultural workers, 20% were daily wage earners, 18% were household workers 14% were unemployed and 10% were businessmen.

Chart 4: Occupation of the study population

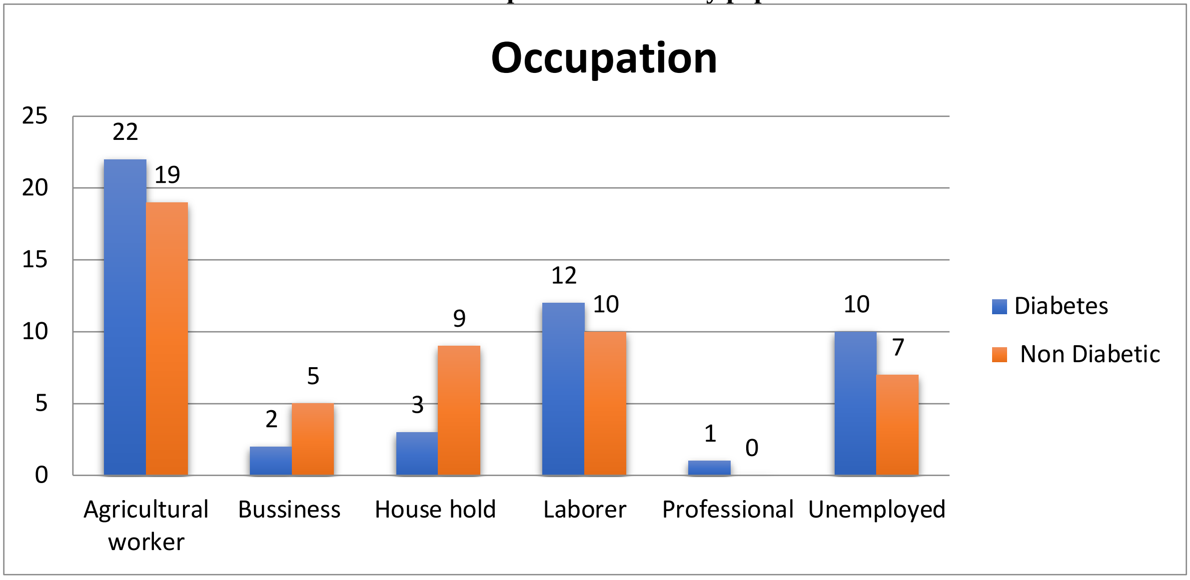

Table 5: Fungal Cultures among study population:

FUNGAL CULTURE	Diabetes	Percentage	Non-Diabetic	Percentage
Negative	23	46.00%	25	50%
Positive	27	54.00%	25	50%
Total	50	100.00%	50	100%

Among diabetics, 54% had positive fungal cultures and rest were negative.Among non-diabetics, 50% had positive and 50% had negative fungal cultures.

Chart 5: Fungal culture in diabetes and non-diabetes

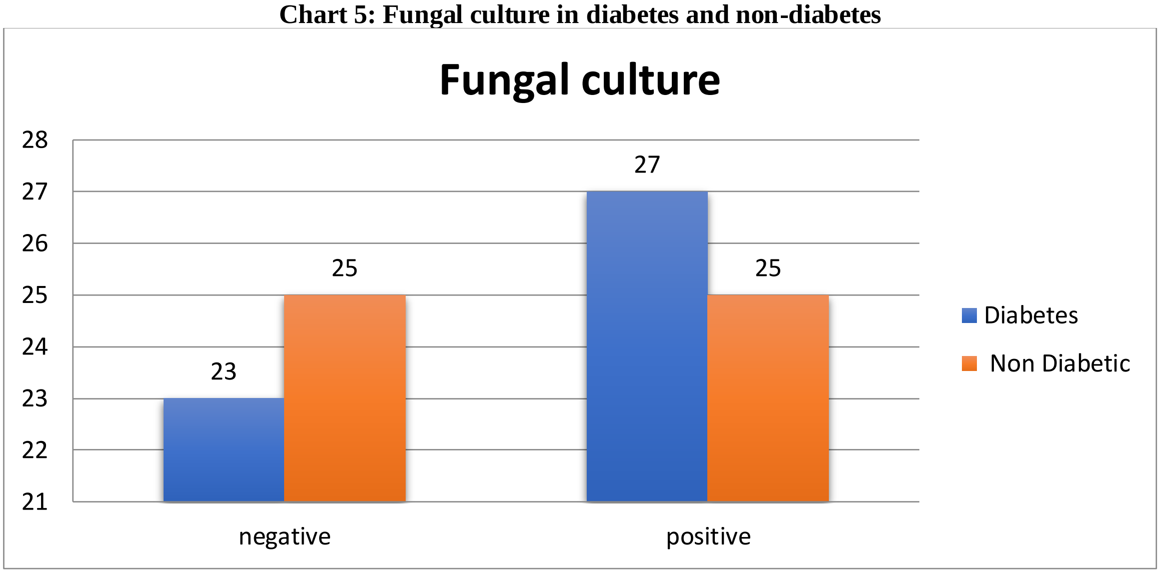

Table 6:KOH mount results among study population

KOH	Diabetes	Percent	Non-Diabetic	Percentage
Negative	25	50.00%	33	66%
Positive	25	50.00%	17	34%
Total	50	100.00%	50	100%

Among diabetics, KOH mount was positive in half of the population.Among non-diabetics, KOH mount was positive in only 34% of the study population.

Chart 6: KOH mount results among study population

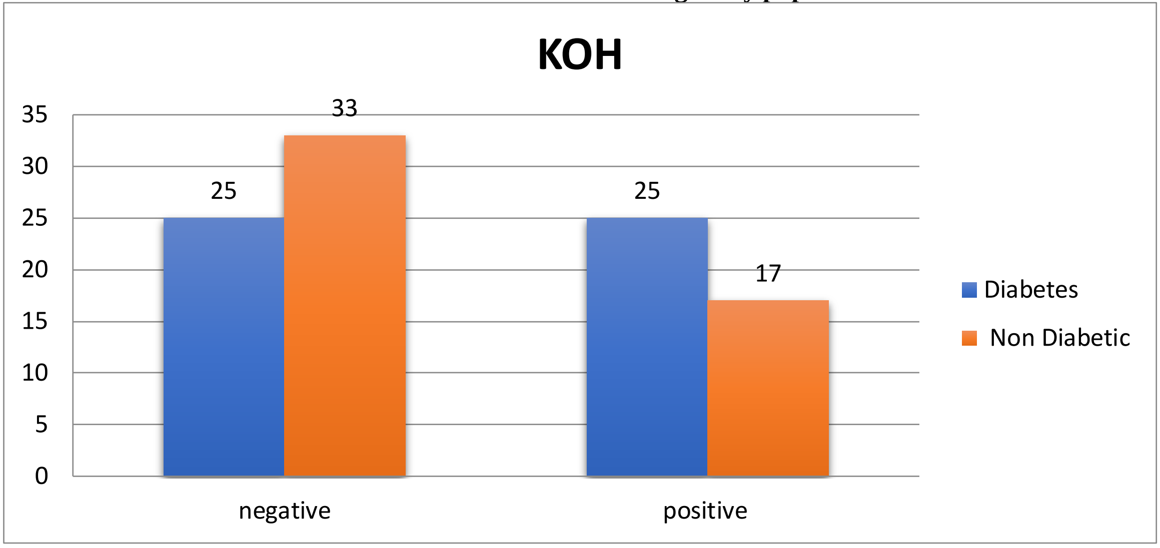

Table 7: Fungal isolates among study population

FUNGI ISOLATED	Diabetes	Percent	Non-Diabetic	Percentage
Aspergillus flavus	4	8%	4	8%
Aspergillus niger	2	4%	4	8%
Aspergilus fumigatus	1	2%	1	2%
Curvularia SPS	2	4%	3	6%
Fusarium SPS	14	28%	12	24%
Unidentified	4	8%	1	2%
None	23	46%	25	50%
Total	50	100%	50	100%

Among diabetics, 28% were Fusarium sps, 8% were Aspergillus flavus, 4% were Aspergillus niger and Curvulariasps each, 2% were Aspergillus fumigatus. 8% of the isolates were unidentified.Among non-diabetics, 24% were Fusarium sps, 8% were Aspergillus flavus and Aspergillus niger each, 6% were Curvulariasps, 2% were Aspergillus fumigatus 2% of the isolates were unidentified.

Chart 7: Fungal culture and KOH mount details in diabetic and non-diabetic patients

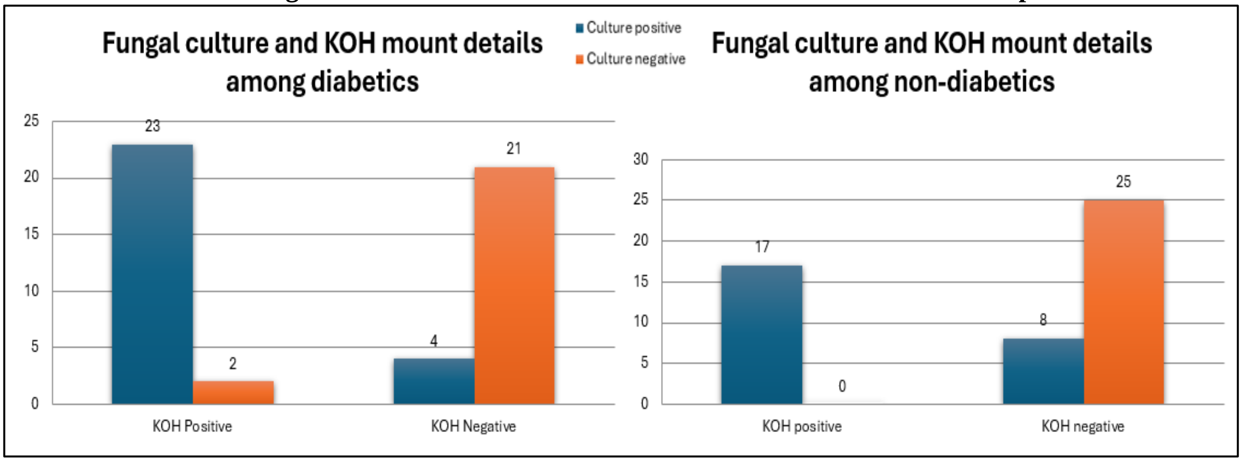

Table 8: Fungal culture and KOH mount details among diabetics:

KOH mount	Fungal culture		Grand Total
	Positive	Negative	
Positive	23	2	25
Negative	4	21	25
Grand Total	**27**	**23**	**50**

Table 9:Fungal culture and KOH mount details among non-diabetics

KOH mount	Fungal culture		Grand Total
	Positive	Negative	
Positive	17	0	17
Negative	8	25	33
Grand Total	**25**	**25**	**50**

DISCUSSION

The results of demographic, risk factor, occupation, fungal organism characterization and other aspects were discussed in brief manner

Age and Gender

In this study, most patients in the diabetic cohort were aged >50 years, with 38% in the 50–59 years group and 36% aged ≥60 years; 22% fell within the 40–49 years group, and 4% in the 30–39 years group. Conversely, most patients in the non-diabetic cohort were aged 30–49 years (38% in the 30–39 years group and 30% in the 40–49 years group), followed by 18% in the 50–59 years group, 8% aged ≥60 years, and 3% in the 20–29 years group. Males predominated in both cohorts (64% vs. 36% females in diabetics; 70% vs. 30% in non-diabetics), likely reflecting greater male engagement in outdoor occupations. The age difference between the two groups is statistically significant with P value of 0.000005

This demographic distribution aligns with established epidemiological trends for diabetes prevalence in older populations and occupational exposure risks associated with infectious keratitis in younger, predominantly male, cohorts. This observed age stratification underscores the differential vulnerability profiles, where older diabetic individuals present with heightened susceptibility, while younger, often occupationally exposed, males in the non-diabetic group exhibit a distinct risk trajectory(9)

Jing et al. conducted a retrospective study comparing the clinical characteristics, treatments, and prognoses of fungal keratitis in patients with and without diabetes, classifying cases into diabetic (n=111) and non-diabetic (n=740) groups (6). Diabetic patients were significantly older (p<0.05) and exhibited a lower male-to-female ratio (p<0.05). Similarly, Bin Wang et al. investigated infectious keratopathy in type 2 diabetes mellitus versus non-diabetic patients, observing no significant difference in sex distribution despite 66.1% of their cohort being male(10) . These findings corroborate the general observation that males are more frequently affected by infectious keratitis, likely due to increased occupational exposure (11), although geographical and socioeconomic factors can influence gender distribution In the study done by Mohod PN et al., to evaluate the incidence of various causes of infectious keratitis in rural central India showed that majority of patients of infectious keratitis were in between 41 and 60 (41%) age group followed by 21–40 (23%) (12).

Risk factor

In the entire study cohort, trauma was the predominant risk factor. Among patients with diabetes, trauma and foreign body exposure were the leading inciting factors (32% each), followed by unknown etiology and prior surgical history. In non-diabetic patients, trauma was most common (40%), followed by foreign body (34%), previous surgery (16%), and unknown factors (5%). This pattern suggests that while trauma remains a universal predisposing factor for infectious keratitis, the specific interplay of additional risk factors, such as systemic comorbidities like diabetes, warrants further exploration for nuanced understanding of disease pathogenesis (13). Jing et al. (6) reported that plant trauma was the primary risk factor in both diabetic and nondiabetic groups.. In a study conducted by Hitesh J. Assudani at C. U. Shah Medical College and Hospital, Surendranagar, Gujarat, the common associated factors identified were injury, diabetes mellitus, contact lens use, and corticosteroid therapy. The study by Hitesh J. Assudani also indicated that trauma due to wooden objects was the leading cause, followed by vegetable matter and stone injury (14). Mohod et al. (12), in their evaluation of the incidence of various causes of infectious keratitis in rural central India, reported that ocular trauma and occupational accidents were the most common predisposing factors among farmers. This is consistent with the global trend where trauma, especially in agricultural settings, frequently precedes infectious keratitis (15). Usha Gopinathan et al. reported that individuals engaged in agriculture-based activities were at a 1.33-fold increased risk of developing microbial keratitis, whereas those with ocular trauma exhibited a 5.33-fold higher likelihood of the condition. These epidemiological data emphasize the critical role of environmental and occupational factors in the pathogenesis of infective keratitis, particularly within agricultural populations (16). Furthermore, younger male agricultural workers have a higher propensity for corneal trauma, which subsequently elevates their risk of developing fungal keratitis (17).

Occupation

In both cohorts, agricultural workers predominated. Among diabetic patients, 44% were agricultural workers, followed by 24% daily wage earners, 20% unemployed individuals, 6% household workers, 4% businessmen, and 2% professionals. In the non-diabetic cohort, 38% were agricultural workers, 20% daily wage earners, 18% household workers, 14% unemployed, and 10% businessmen. This occupational profile aligns with the elevated risk of trauma-induced keratitis among agricultural workers, particularly in rural settings (12,15). This predisposition is further amplified by exposure to vegetative matter, a common source of ocular foreign bodies in agricultural environments, significantly increasing the likelihood of fungal keratitis (18). Bin Wang et al. conducted a comparative analysis of the clinical characteristics of infectious keratopathy in patients with type 2 diabetes mellitus versus non-diabetic individuals, revealing a statistically significant difference in occupational distribution between the two groups (10). Mohod PN et al. (12), in their evaluation of the incidence of various causes of infectious keratitis in rural central India, reported that ocular trauma and occupational accidents were the most common predisposing factors among farmers. Researchers reported that individuals engaged in agriculture-based activities exhibited a 1.33-fold increased risk of developing microbial keratitis, whereas those with ocular trauma demonstrated a 5.33-fold higher likelihood of the condition (16).

Culture positive

In the present study, among diabetics, 54% had positive fungal cultures and rest were negative. And among non-diabetics, 50% had positive and 50% had negative fungal cultures. Overall, 52% were positive for cultures. This rate is comparatively lower than the 60.5% culture positivity reported by Mohod et al., highlighting potential variations in etiological prevalence or diagnostic methodologies across different regions (19). The observed discrepancies in culture positivity rates underscore the need for standardized diagnostic protocols and robust epidemiological surveillance to accurately ascertain the true burden of fungal keratitis (20). In a research study cultures were positive in 811 patients. Among these culture-positive cases, 509 exhibited pure fungal infections, 184 pure bacterial infections, and 114 mixed fungal and bacterial infections (21). Bharathi et al while evaluating the clinico-demographical profile of keratomycosis, culture growth was obtained from 80 of 209 keratitis cases examined. Among these 80 culture-positive cases, fungi were isolated in 77.5% and bacteria in 22.5%. Routine surveillance of fungal keratitis is essential to monitor existing and emerging pathogen patterns and to avert unwarranted antimicrobial therapy(22). Gopinathan et al. reported that, of 5897 suspected cases of microbial keratitis, 3563 (60.4%) were culture-proven (20). In a study by Srinivas Jampala in Kochi, aimed at assessing the frequency of infective keratitis along with its etiology, antimicrobial sensitivity patterns, risk factors, and clinical outcomes, culture-proven cases accounted for 61.2%, exhibiting pure bacterial, pure fungal, and mixed growth patterns. Gupta and Rishi documented a higher prevalence of fungal keratitis (37 cases) relative to bacterial keratitis (28 cases). Similarly, Manikandan et al. observed culture positivity in 82.2% of corneal scrapings, identifying bacteria, fungi, and parasites (21). Furthermore, an earlier study revealed that 68% of 96 samples tested positive for culture, with 37 of these cases indicating fungal growth and 28 bacterial (23). These diverse findings highlight the significant variability in microbial etiology across different geographical regions and diagnostic settings, underscoring the importance of localized epidemiological studies for effective clinical management (24,25). Although culture methods remained the cornerstone of fungal keratitis diagnosis, most studies sensitivity of culture methods in detecting fungal keratitis can be as low as 50%, and culture-negative cases may still demonstrate fungal filaments through microscopic examinationand can be diagnosed as fungal keratitis(26).

Fungal organism and characteristics

In the present study, fungal cultures were positive in 54% of diabetics (with the remainder negative) and 50% of non-diabetics (evenly split between positive and negative), yielding an overall positivity rate of 52%. KOH mounts were positive in 50% of diabetics and 34% of non-diabetics. Among diabetics, the fungal isolates comprised 28% *Fusarium* spp., 8% *Aspergillus flavus*, 4% each *Aspergillus niger* and *Curvularia* spp., 2% *Aspergillus fumigatus*, and 8% unidentified. Among non-diabetics, they included 24% *Fusarium* spp., 8% each *Aspergillus flavus* and *Aspergillus niger*, 6% *Curvularia* spp., 2% *Aspergillus fumigatus*, and 2% unidentified.

Assudani et al., in a study conducted at C. U. Shah Medical College and Hospital, Surendranagar, Gujarat, involving 100 patients, reported prevalences of 14% for mycological keratitis and 13% for bacterial keratitis; among the 14 fungal isolates identified, 8 were *Aspergillus flavus* and 6 were *Aspergillus niger*. Basak et al. similarly identified *Aspergillus* spp. as the predominant fungal pathogen (373 isolates), followed by *Fusarium* spp. (132 isolates) among positive cultures. Gupta et al., evaluating the clinico-demographical profile of keratomycosis, documented a spectrum including *Aspergillus flavus*, *Fusarium solani*, *A. fumigatus*, *Candida albicans*, and others. Joshi et al. reported *Aspergillus fumigatus* as the most common isolate, while Ranjini et al., assessing infectious keratitis at a tertiary eye care hospital, found *Fusarium* spp. most frequent (36 cases), followed by *Aspergillus* spp. (13 cases). Mohod et al. (12) observed a fungal keratitis prevalence of 59.09% in rural central India, exceeding bacterial and viral etiologies. Gopinathan et al. (20) identified *Fusarium* spp. as the leading fungal cause, whereas Jampala studying keratitis frequency in Kochi, noted *Candida* spp. as predominant. Mudhol et al. found *Fusarium* and *Aspergillus* spp. equally responsible for most fungal infections, consistent with Manikandan et al. (21), who predominantly isolated these genera. These regional variations underscore the intricate epidemiological landscape of fungal keratitis, necessitating localized surveillance to inform diagnostic and therapeutic strategies effectively. Despite this variability, a consensus exists that direct microscopic examination of corneal scrapes, particularly with stains like potassium hydroxide and Calcofluor White, remains a critical initial diagnostic step due to its rapid turnaround time and high sensitivity in detecting fungal elements (27,28). Further studies from various regions of India indicate a diverse spectrum of fungal pathogens, with some reporting high positivity rates for Aspergillus and Candida species, particularly in central India, while others emphasize Fusarium species prevalence in eastern coastal states (29). Indeed, studies from India further corroborate the predominance of *Aspergillus* species, especially *Aspergillus fumigatus* and *Aspergillus flavus*, as significant etiological agents, while *Candida albicans* also features prominently in some regional analyses (30,31). Conversely, in North and Northeast India, *Aspergillus* species were found to be the predominant causative agents, whereas *Fusarium* species were more frequently isolated in the southern regions (32,33). This geographical variation in prevalent fungal species highlights the influence of environmental and climatic factors on the epidemiology of fungal keratitis (34).

Role of diabetes in fungal keratitis the specific distribution of fungal species observed in diabetic versus non-diabetic individuals suggests a potential impact of glycemic control on the pathogenic landscape of fungal keratitis. This highlights the imperative for further investigation into how hyperglycemia and compromised immune responses in diabetic patients may influence fungal colonization and virulence, thereby shaping the predominant fungal species encountered in this population. Furthermore, varied environmental factors across geographical regions contribute to the heterogeneity in fungal species distribution, with some studies indicating Fusarium species as predominant in South India while others from Saudi Arabia and Gujarat, India, report Aspergillus species as more prevalent (11). Such regional variations in etiological agents necessitate localized epidemiological surveillance to inform targeted antifungal treatment strategies and optimize patient outcomes.

CONCLUSION

In conclusion, systemic factors like diabetes and environmental influences shape infectious keratitis etiology. Geographical variations in dominant fungi- *Fusarium* spp. in tropical regions versus *Aspergillus* spp., elsewhere, require region-specific diagnostics and therapies. For instance, *Fusarium* spp. predominate in tropics, while *Aspergillus* and *Candida* spp. are common elsewhere. Localized epidemiological studies guide empirical treatments and outcomes. Clinicians must incorporate regional patterns, especially amid rising diabetes. Ongoing surveillance of pathogens and susceptibilities in diabetics is essential for targeted guidelines, given fungal keratitis' burden in resource-limited settings. Prospective molecular studies are recommended for species-level insights across India.

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