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Investigating fungi associated with human corneal infections among patients in Ramadi

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Abstract

Fungal keratitis is a major contributor to ocular morbidity. The gradual progression, overlapping characteristics, diagnostic complexity, and potential consequences of fungal keratitis present a significant clinical problem. The rising trend can be related to the utilization of contact lenses, non-judicial corticosteroids, and vegetative trauma. Prompt identification and treatment are essential for effectively managing it. Understanding the pathological progression and clinical features of fungal keratitis is crucial for promptly diagnosing and treating the condition, leading to a decrease in ocular complications. A total of 50 specimens from infected eyes were collected and studied microbiological 30 Positive fungal growths were selected from September to November 2023 from patients admitted in different hospitals in Ramadi city. Patients and private clinical were subjected to direct examination by 10% KOH mount, gram stain, and culture PDA, SDA. Eye infections in relation to sex revealed that female cases 18(62.07%) were higher than those of males 11(37.93%). Infection in relation to age group indicated that those of age more than 40 years 14(48.28 %) were the most susceptible. Also, infections in relation to the geographical area of patients revealed that the rural 15 (55.56 %) were higher than Patients living in a city 12(44.44 %). Distribution of isolates fungal eye infections according to growth rate a total of 30 Positive fungal growth, species related to the type of fungus were identified, of which *Asporgillus nigar* 20 (66.67%) were the most prevalent, while Fusarium *oxysporum* recorded 7 (23.33 %), the lowest percentage recorded Fusarium *solani* 3 (10 %). Mycotic keratitis is a severe issue that typically occurs after an injury to the cornea. It necessitates prompt diagnosis and identification of fungal agents in order to administer appropriate therapy and avoid catastrophic outcomes. The species *Asporgillus nigar* was the most common fungal isolate.

Introduction:

Infections of the eye caused by fungi are extremely uncommon. However, there are a great number of fungi that have been reported to infect the eye in one of three ways: either through direct introduction through trauma or surgery, through extension from infected surrounding tissues, or through hematogenous dissemination to the eye. Fungal keratitis is

caused by septate filamentous saprophytic fungus in the great majority of cases diagnosed with the condition. It has been suggested that a number of fungi, such as species of *Acremonium*, *Aspergillus*, *Candida*, *Paecilomyces*, dermatophytes, *Rhinosporidium seeberi*, and *Sporothrix schenckii* , are responsible for the development of dacryocystitis or chronic granulomatous dacryocystitis [1].

Two of the most common causes of fungal keratitis are the fungi *Aspergillus* and *Fusarium*. Although it is associated with a higher incidence of problems, *Aspergillus* has a superior response to antifungal medicines [2]. Common fungal isolates were obtained from corneal scrapings taken from patients who had a clinical suspicion of keratitis. It is most commonly related to contact lens-induced keratitis in which *fusarium* is present. At an early stage in the evolution of *Fusarium* keratitis, it is essential to recognize the condition, and appropriate actions should be taken in order to reduce the amount of ocular morbidity that occurs. There have been many cases of *Fusarium* keratitis that have been documented in conjunction with the use of CL and ReNu CL solution (Bausch & Lomb) for maintenance of lenses. This might be related to inappropriate use habits and inadequate hygiene practices when it comes to contact lenses [3]; some of these individuals needed to have emergency penetrating keratoplasty because they were also suffering from serious complications related to their keratitis.

There are different *Fusarium* species and complexes responsible for keratitis: Fusarium solani species complex, Fusarium oxysporum species complex, and *Gibberella fujikuroi* species complex. *Fusarium solani* species complex is the most common [4]. Keratitis typically manifests as ocular pain, a sensation of a foreign object in the eye, and visual blurring [5]. The affected eye will exhibit erythema, and the patient may experience conjunctival injection. The intense inflammatory response following an infection typically leads to a significant volume of ocular secretions. However, in cases of fungal keratitis, the secretions are usually minimal compared to other types of microbial infections [6]. The diagnosis of fungal keratitis begins by having a high level of suspicion based on clinical observations and the presence of risk factors. The diagnosis is strongly supported by the presence of clinical symptoms that strongly indicate the condition, as well as the isolation of the fungus from a sample taken from the cornea. Antibiotic resistance serves as a valuable clinical indicator for diagnosis. The gold standard for diagnosing fungal infections is the direct microscopic inspection of the cornea followed by culturing a sample from the cornea [7].

Although there are many previous studies, this study is considered the first of its kind to isolate and diagnose fungi associated with human eye infections in Ramadi city patients

Materials and Methods

The current study included 50 patients (30 of whom were diagnosed with infectious corneal ulcers) who were inpatients at the Eye Center at Ramadi City Hospital and private clinics between September and November 2023, as the main eye referral center.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of the First Affiliated Hospital of Ramadi city (No.:22-3.0).

A- Clinical Diagnosis

A detailed history from each patient with a clinical diagnosis of corneal ulcer was recorded, including the age, sex, occupation, onset of symptoms, predisposing risk factors, previous treatment methods, and visual outcomes. Slit-lamp biomicroscopy was utilized in order to carry out an ocular examination on both of the patient's eyes. Evidence was gathered regarding the visual acuity at the time of presentation, symptoms, as well as the amount and depth of the stromal infiltrate. In addition to this, the depth of the lesion, the presence or absence of hypopyon, and the condition of the anterior chamber were all examined.

Isolation of Fungi and Sampling of Infected Eye Specimens

A definitive diagnosis of fungal keratitis was made if: (I) corneal scrapings incubated with potassium hydroxide (KOH) and examined on wet mounts revealed fungal elements in smears; (II) fungus grew in more than one medium when corneal scrapings were subjected to fungal culture and strain identification [8].

laboratory procedures for Identification of Fungi

Aseptic corneal scrapings were obtained from the ulcer's leading edge or base using a disposable microblade. A microscopic examination of each scraping was conducted to detect the presence of fungi using lacto phenol cotton blue staining. The fungal culture was deemed positive using direct microscopy, and the isolates were identified by analyzing their macroscopic and microscopic colonial morphology .

The corneal scrape was examined under a microscope using a 10% potassium hydroxide (KOH) mount. BA and CA plates were incubated at 37°C for seven days. The plates were discarded if no growth was observed within this 7-day period. On the other hand, SDA plates were subjected to incubation and evaluated based on their biochemical properties.

Sabouraud's dextrose agar was used to isolate pathogenic fungi from the clinical specimens. The composition of this medium is (g/l): peptone, 10; dextrose, 40; agar, 20; pH 5.2- 5.6. To prevent the growth of bacteria and saprophytic fungi, chloramphenicol was added at 250 mg/l, and cyclohexamide was added at a concentration of 0.5 g. The plates treated with a small amount of a substance to induce immunity were placed in a controlled environment with a temperature of 27°C for two weeks. (4) Saprophytic fungi were isolated using Czapek's glucose agar. The composition of the solution is as follows (g/l): KH_2 PO_4, 1; $MgSO_4.7H_2O$, 0.5; glucose, 10; agar, 15; with a pH of 5.6. Bacteriostatic agents, such as rose bengal (1:15000) and chloramphenicol (250 mg/l), were included in the mixture. The plates were placed in an incubator at a temperature of 27°C for one week after being inoculated. **Inoculation procedure:** The plates were inoculated by gently streaking cotton swabs over the agar surface in rows of C-shaped streaks, ensuring that the agar surface was not pierced [9]. Growth on the C-streaks is seen as meaningful, but growth outside the C-streaks is likely attributable to contamination. The scrapes were injected directly into the center of the plates.

The Sabouraud's dextrose agar was incubated at 25°C and 37°C for four weeks. The culture was assessed once during the first week and twice every week for the subsequent three weeks. The culture was discarded if no development was observed after four weeks of

incubation. Their distinctive morphological characteristics facilitated the identification of any growth achieved.

Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) comprises dried potato infusions and dextrose. The potato infusion is a nutritional source that supports the abundant growth of most fungi, while dextrose is a stimulant for growth. The agar in the medium functions as the gelling agent. The inclusion of drugs such as tartaric acid, chloramphenicol, and chlortetracycline has enhanced the modification of PDA. Introducing tartaric acid (TA) into the medium reduces the pH to 3.5, thereby suppressing bacterial growth. Chloramphenicol is a selective agent that hinders the excessive growth of competing microorganisms in mixed specimens while allowing for the specific separation of fungus. Chlortetracycline is used in PDA to assess the presence of yeast and mold in cosmetic goods. The materials and their quantities for PDA are as follows: Potatoes (infusion from): 200.0 g/l, Dextrose: 20.0 g/l, Agar: 15.0 g/l, Distilled water: 1000 ml. The final pH of the mixture at 25°C is 5.6±0.2.

Statistical analysis

Use statistical software SAS- Statistical Analysis System, In analyzing data to compare the significant differences between levels of different factors, The significant differences between the means ere compared with the Chi-square test.2 And on (Chi-Square-χ Probability level 0.05 and 0.01 [10].

Results and Discussion

Isolation and identification of *Fusarium solani* and *Aspergillus* on SDA

The results obtained in this study indicated the growth of endophytic isolates of the fungus *Fusarium solani* and *Aspergillus* on a selective medium of Sabouraud Dextrose Agar (SDA) containing the antibiotics streptomycin 10 µg/dL and chloramphenicol 25 µg/dL. The results showed that two types of fungi, *Fusarium solani* and *Aspergillus*, were identified from the samples taken from patients in the period between September to November 2023, as shown in Figure 1.

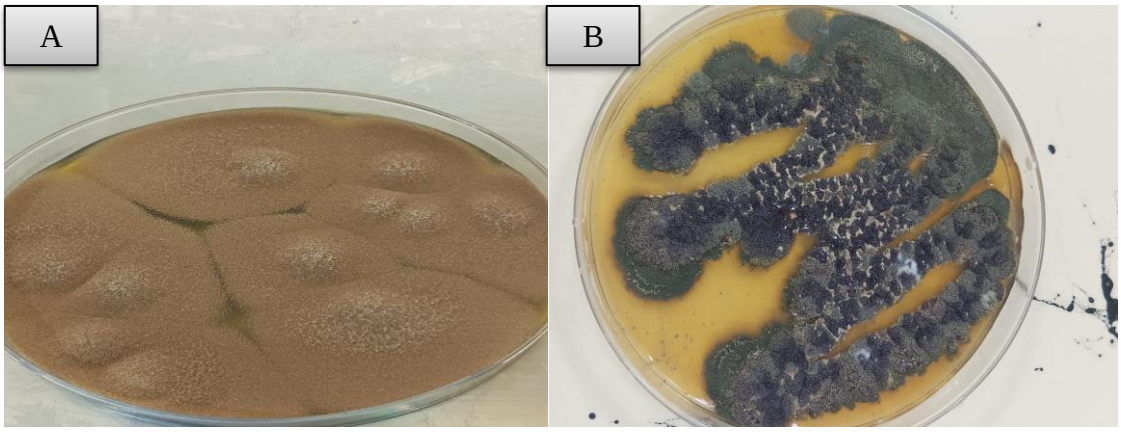

Fig. 1 Colony of (A) *Fusarium solani* and (B) *Aspergillus* on SDA.

Microscopic diagnosis

The colonies exhibited a whitish, woolly, and cottony appearance. The Lactophenol Cotton Blue mount examination showed hyaline branched septate hyphae, characterized by long monophialidic conidiogenous cells. This characteristic helps to distinguish *Fusarium solani* from *Fusarium oxysporum* [11].

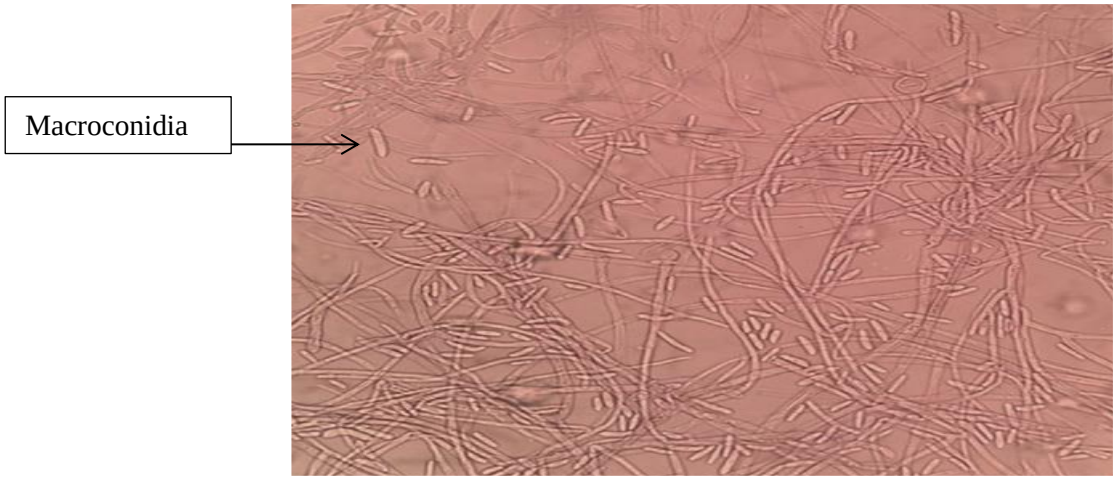

Fig. 2 microscopic apperance of *Fusarium solani* (40x).

Distribution of isolates fungal eye infections according to growth rate

The assessment fungal growth rate in patients with eye infections is presented in Table (1), where it is seen that the Positive fungal growth population exhibited the greatest level of 30 (60%), while negative fungal growth was recorded at 20 (40%). A statistically non-significant difference was seen between fungal growth rates when the Chi-Square -$\chi 2$ test was conducted, with a p-value of 0.1570.

Table 1: Distribution of isolates fungal eye infections according to growth rate

Fungal growth rate	**No.**	**%**
Positive growth	30	60.00
Negative growth	20	40.00
Total	50	100 %
Chi-Square -$\chi 2$ (P-value)	0.1570	

Fusarium solani and *Aspergillus* are the most common genus of filamentous fungus that cause eye infections, with a high occurrence rate. In our study, fungi were detected in 60% (30 out of 50) different of study Shivaji S *et al*., [12], which recorded that 25.9% (29 out of 112) corneal scrapping samples [13], also discovered a comparable pattern of fungal isolation from ocular ulcers. The findings of this study align with prior research [9, 36-39], predominantly based on observations from the afflicted eye. This can be attributed to various factors, such as the use of contact lenses and the cleaning solutions employed for them.

Distribution of isolates Fungal eye infections according to age group

The assessment of isolates Fungal eye infections according to age group is presented in Table (2), where it is seen that the < 40 age exhibited the greatest level 14(48.28 %), The lowest incidence of infection, at 3-10 age and 10-20 age recorded 3 (10.34%). While 20-30

age and 30-40 age recorded 4(13.79%), 5(17.24%) respectively. A statistically significant difference was seen in age groups when the Chi-Square -χ2 test was conducted, with a p-value of 0.01.

Table 2: Distribution of isolates Fungal eye infections according to age group

Age	Positive growth%	Negative growth%	Total %
3-10	3 (10.34%)	3(14.29%)	6(12.00%)
10-20	3 (10.34%)	4(19.05%)	7(14.00%)
20-30	4(13.79%)	5(23.81%)	9 (18.00%)
30-40-	5(17.24%)	5(23.81%)	10(20.00%)
< 40	14(48.28 %)	4(19.05%)	18(36.00 %)
Total	29	21	50
Chi-Square -χ2 (P-value)	(P≤0.01) **.		

Eye fungi can infect all age groups, whether children under six, young people, or even older adults. In the current study, the results indicate that the age group older than 40 years was more susceptible to infection with eye fungi, and this could be due to several reasons, including the genetic factor, a weak immune system, chronic diseases, and malnutrition, and the results obtained in this study are consistent with the study that Conducted by Thornton *et al*[14] .

Distribution of isolates fungal eye infections according to geographical area

The assessment of isolated Fungal eye infections according to geographical area is presented in Table (3), where it is seen that the rural area exhibited the greatest level of 15 (55.56 %), The lowest incidence of infection, while city area recorded 12(44.44 %). A statistically non-significant difference was seen in the geographical area when the Chi-Square -χ2 test was conducted, with a p-value of 0.563.

Table 3: Distribution of isolates fungal eye infections according to geographical area

Residence area	Positive samples	Negative samples	Total %
rural area	15 (55.56 %)	7 (30.43%)	22(44.00 %)
city	12(44.44 %)	16 (69.57 %)	28 (56.00 %)
Total	27	23	50
Chi-Square -χ2 (P-value)	0.563NS		

Many confounding factors such as environment, smoking, eating disorders, and immune status of the host, antibiotics play an important role in eye fungal infection. In our study, we recorded results 15 (55.56 %) of patients were lives in rural areas are most incidence of infection from patients were lives in city areas. In study conducted by [15] reported that Farmers were the most common patients with fungal keratitis.

Aspergillus species are frequently identified as the second most prevalent cause of mycotic keratitis. However, most research conducted in India has consistently identified *Aspergillus* as the most prevalent cause of fungal keratitis. In the second investigation, the occurrence of dematiaceous fungi (Curvularia, Alternaria, Cladosporium, Aureobasidium pullulans) was determined to be 24.13%. Previous research has identified dematiaceous fungi as the third most prevalent causative agent of fungal keratitis[16], Male individuals between the ages of 30 and 50 who engage in outdoor agricultural activities are at a higher risk of sustaining ocular injuries caused by plant matter. This may explain the higher prevalence of such injuries among males in this age group. Yeasts and molds are significant fungal pathogens that cause fungal keratitis [12]

Distribution of isolates fungal eye infections according to relationship between other diseases and the type of fungus spp.

The assessment of isolates Fungal eye infections according to the relationship between other diseases and the type of fungus spp.is presented in Table (4), where it is seen that the *Fusarium oxysporum , Fusarium solani* and *Asporgillus nigar* with other diseases (Hypertension, Diabetics and Chronic allergy) a statistically non-significant difference was seen when the Chi-Square -$\chi 2$ test was conducted, with a p-value of 0.174 and 0.139 respectively. Fungal infections in the cornea were more frequently reported in patients with diabetes compared to patients with chronic pressure and chronic allergy diseases.

Table 4: The rate of fungal infection according to the relationship between the disease and the type of fungus

Fusarium oxysporum , Fusarium solani ***%***	***Asporgillusnigar*** ***%***	**Diseases**
1(9.09 %)	6 (31.58 %)	Hypertension
6 (54.55%)	10(52.63 %)	Diabetics
4 (36.36%)	3 (15.79%)	Chronic allergy
11(36.67 %)	19(63.33 %)	Total
NS 0.174	NS 0.139	Chi-Square -$\chi 2$ (P-value)
Total no. 30		

Fungi that cause fatal disseminated infections are usually opportunistic, taking advantage of weakened immune systems due to primary immunodeficiency, AIDS, hematological malignancies, or the use of strong immunosuppressive therapies that deplete microbial immunity, preexisting ocular surface diseases, and systemic disorders such as diabetes mellitus. The current study agrees with many previous studies in that there is no effect linked between diseases such as diabetes and chronic diseases with fungi that infect the eye [14, 17].

Distribution of isolates fungal eye infections according to types of fungus spp.

The assessment of isolates Fungal eye infections according to relationship **types of fungus spp.**.is presented in Table (5), where it is seen that *Asporgillus nigar* exhibited the greatest level 20 (66.67%), while *Fusarium oxysporum* and *Fusarium solani* recorded that 7 (23.33%) and 3 (10.00%) respectively.

A statistically significant difference was seen in **types of fungus spp** when the Chi-Square -χ2 test was conducted, with a p-value of 0.0003.

Table 5: Distribution of isolates fungal eye infections according to type of fungus spp.

fungus	**No.**	**%**
Asporgillus nigar	20	66.67
Fusarium oxysporum	7	23.33
Fusarium solani	3	10.00
Total	30	100
Chi-Square -χ2 (P-value)	0.0003	

In this study, *Asporgillus nigar* was the most frequent isolate, followed by Fusarium has also been found to be the predominant species in Ramadi city. However, in Northern India, Nepal, and Bangladesh, *Aspergillus* is the most frequent cause of fungal keratitis [15]. The most common fungus reported in China is Fusarium, which accounts for 58.7% of cases, different to the findings of our study [8]. This phenomenon may be explained by the differences in demographic characteristics, climate, and the natural environment. Filamentous fungi are more frequently encountered than yeasts in tropical and subtropical regions. Fusarium grows in cornea horizontally, diagonally and vertically, and the hyphae are easy to spread and multiply around the lesion while *Aspergillus grow* in cornea diagonally and vertically [18].

Distribution of isolates fungal eye infections according to gender

The assessment of isolates Fungal eye infections according to **gender** is presented in Table (6), where it is seen that Female exhibited the greatest level 18(62.07%), while Male recorded that 11(37.93%). A statistically significant difference was seen in gender when the Chi-Square -χ2 test was conducted, with a p-value of 0.193.

Table 6: Distribution of isolates fungal eye infections according to gender

Sex	**Positive samples**	**Negative samples**
Male	11(37.93%)	14(66.67%)
Female	18(62.07%)	7(33.33%)
Total	29(58.00%)	21(42.00%)
Chi-Square -χ2 (P-value)	NS 0.193	

The present results agreement with the result of [19] found that fungal keratitis is more frequent in females than males, resulting in a longer recovery duration [19], also found that *Fusarium* and *Aspergillus* are frequently identified in corneal scrapings, specifically linked to contact lenses. An eye in good health is relatively resistant to fungal infection. However, ocular damage can create a favorable environment for fungal pathogens to enter the eye. Fungal keratitis has a very sluggish course with severe sequelae that might vary from corneal

scarring to perforation and, eventually, loss of eyesight. The current investigation has identified *Aspergillus* niger as the predominant causative agent of corneal ulcers, which aligns with previous research findings [20]. Fungal isolation from ocular ulcers was notably more prevalent in female patients [21]. The incidence of fungal keratitis in our study was similar to that reported in research results from different parts of the world, indicating that the incidence of fungal keratitis ranges from 6% to 56% [15] in females. Most of the cases in the world can be attributed to unhygienic contact lens use.

Conclusions

In summary, this study focused on the epidemiological and etiological data obtained from a large cohort of patients with fungal keratitis in Ramadi City and provides significant insights into the understanding of this potentially devastating corneal disease. The *Aspergillus* species was the most commonly isolated pathogen; agricultural activity was the principal causative factor. We hope that our study may help our colleagues diagnose and properly treat this disease and achieve a better outcome for patients.

Conflicts of interest

There are no conflicts of interest.

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دراسة الفطريات المرتبطة بالتهابات القرنية البشرية بين المرضى في مدينة الرمادي

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Assporgillus nigar، فيوزاريوم أوكسيسبوروم، فيوزاريوم سولاني، التهابات العين، المساعد الرقمي الشخصي، SDA

معلومات المؤلف

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الموبايل:

الخلاصة:

التهاب القرنية الفطري هو المساهم الرئيس في مراضة العين. يمثل التقدم التدريجي والخصائص المتداخلة والتعقيد التشخيصي والعواقب المحتملة لالتهاب القرنية الفطري مشكلة سريرية كبيرة. يمكن أن يكون الاتجاه الصاعد مرتبطًا باستخدام العدسات اللاصقة والكورتيكوستيرويدات غير القضائية والصدمات الخضرية. يعد التشخيص والعلاج الفوري ضروريين لإدارة المرض بشكل فعال. يعد فهم التطور المرضي والسمات السريرية لالتهاب القرنية الفطري أمرًا بالغ الأهمية لتشخيص الحالة وعلاجها بسرعة، مما يؤدي إلى انخفاض المضاعفات العينية. جمع ما مجموعه 50 عينة من العيون المصابة ودراستها الميكروبيولوجية 30. تم اختيار نمو فطري إيجابي خلال شهري سبتمبر إلى نوفمبر 2023 من المرضى المقبولين في مستشفيات مختلفة في مدينة الرمادي، وتم إخضاع المرضى والعيادات الخاصة للفحص المباشر بنسبة 10٪ من KOH وصبغة جرام وصبغة جرام. ثقافة المساعد الشخصي الرقمي، SDA. أظهرت التهابات العين بالنسبة للجنس أن حالات الإناث 18 (62.07%) كانت أعلى من حالات الذكور 11 (37.93%). أشارت الإصابة بالنسبة للفئة العمرية إلى أن الأشخاص الذين تزيد أعمارهم عن 40 سنة 14 (48.28٪) كانوا الأكثر عرضة للإصابة. وأظهرت الإصابات بالنسبة للمنطقة الجغرافية للمرضى أن الريف 15 (55.56%) كانوا أعلى من المرضى الذين يعيشون في المدينة 12 (44.44%). توزيع عزلات التهابات العين الفطرية حسب معدل النمو الكلي 30 نمو فطري إيجابي، وتم تحديد الأنواع المرتبطة بنوع الفطريات وكان 20 *Assporgillus nigar* (66.67%) الأكثر انتشاراً، في حين سجل 7 *Fusarium oxysporum* (23.33%)، أقل نسبة سجلت *Fusarium solani* 3 (10%). التهاب القرنية الفطري هو مشكلة خطيرة تحدث عادة بعد إصابة القرنية. يتطلب التشخيص السريع وتحديد العوامل الفطرية من أجل إدارة العلاج المناسب وتجنب النتائج الكارثية. كان النوع *Assporgillus nigar* هو أكثر العزلات الفطرية شيوعًا..