

# Frequency of the Most Common Fungal Infection in Biopsies of Patients, Presenting to Pathology Department King Edward Medical University Mayo Hospital, Lahore, Keeping the Pas Stain as Gold Standard

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## Abstract

**Background:** Fungi are eukaryotic species that live in the form of yeast and molds. They are eukaryotic, but they have a cell wall. It is possible to distinguish mycosis into four types; skin, subcutaneous, systemic and opportunistic.

**Objectives:** To determine the frequency of the most common fungal infection.

**Methods:** This retrospective study was performed in histopathology department of King Edward Medical University by reviewing previous records. The study was performed during November 2020 to May 2021. 1295 were the biopsies with chronic nonspecific inflammation. 34 biopsies were diagnosed with superadded fungal infection. These patients were those who presented to outdoor, general surgery, plastic surgery, maxillofacial, burn, eye unit and ICU.

**Results:** In our study, 18 (52.9%) patients were males and 16 (47.1%) were females, the total patients with fungal infection were 34. 23 out of 34 (67.6%) patients were diagnosed with mucormycosis, the second most frequent fungal infection reported was Candidiasis with a frequency of 5 out of 34 (14.7%), and then aspergillosis with a frequency of 4 (11.8%).

**Conclusion:** It is concluded that the most frequent fungal infection reported in Mayo Hospital is Mucormycosis, keeping the PAS stain as the gold standard, with a frequency of 23 out of 34, and a percentage of 67.6%.

**Keywords:** *Fungus, mucormycosis, aspergillosis, candidiasis, PAS stain.*

## INTRODUCTION

Fungi are eukaryotic species that live in the form of yeast and molds. They are eukaryotic, but they differ from plants and animals because, unlike plants, they have a cell wall. The fungal cell wall is made up of chitin, so fungi are resistant to such drugs [1]. In comparison to human cell membranes that contain cholesterol, the fungal cell membrane contains ergosterol [2]. Two forms of fungi exist; yeast and molds. Yeasts are single cells and create asexual budding. Molds form a mycelium and are long filaments (hyphae). Some hyphae form transverse walls, that is, aseptate hyphae, while others form nonseptate hyphae. *Candida Albicans* is a part of the natural flora of humans [3]. In the processing of essential fungi are used [4]. The development of granulomas is the response to infection with several fungi [5].

It is possible to distinguish mycosis into four types; skin, subcutaneous, systemic, and opportunistic [6]. Pulmonary pathogens include *Histoplasma capsulatum* and *Aspergillus fumigatus* [7]. Opportunistic mycoses are due to fungi that in most immunocompetent individuals do not cause disease but can induce disease in those with compromised host defences [8].

The cause of cryptococcal meningitis is *Cryptococcus neoformans*. Often predisposed to fungal infection are patients with immunosuppression, poorly controlled diabetes, burns, neutropenia, HIV and pancreatitis [9]. Occupational exposures are associated with blastomycosis. Renal transplantation, a major cause of mortality and morbidity is invasive fungal infections [10].

*Mucor* and *Rhizopus* are saprophytic molds, causing mucormycosis. They proliferate in the walls of the blood vessels, particularly the sinuses, intestines or lungs, causing necrosis and tissue infarction. Diabetic patients are prone to rhinocerebral mucormycosis, in which hyphae are produced by mold spores in the sinuses that infiltrate the blood vessels that supply the brain. Fungal meningitis and bloodstream infections are less common than skin and lung infections and can be lethal. As the signs of yeast infection may be identical to those of other illnesses, there is often a delay in diagnosis as well as treatment [11].

Gomori Methenamine Silver, GMS and Periodic Acid-Schiff, PAS are the two most common stains used for fungus identification [12, 13].

According to the Journal of Clinical Medical Research 2015, in solid organ transplant recipients, average mortality due to invasive fungal infections varies from 25% to 80% [14].

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The survey in Egypt in June 2017 found that the most prevalent infection was onychomycosis (64.8 percent of cases), and tinea capitis (17.6 percent) [15].

In Korea, the annual prevalence of all-type mycosis increased from 6.9% in 2012 to 7.4% in 2017, based on the National Health Insurance of South Korea data. The prevalence of dermatophytosis was highest (5.2%) among the particular classes, followed by opportunistic mycosis (1.7%) and superficial mycosis (0.2%) [16].

The majority of aspergillus infections (78 percent) are restricted to the lungs [17]. In 45 percent and 39 percent of cases, CNS involvement and disease confined to the lungs were observed among cryptococcosis cases, respectively [18]. Disseminated multi-organ infections include blastomycosis, coccidioidomycosis, and histoplasmosis [19].

Vegetable material trauma is thought to be a particular threat to fungal infection of the cornea [20]. AML and ALL patients developed candidaemia at incidence rates of 2-3% and 4-5%, respectively, in an Italian study [21]. Invasive candidiasis in premature neonates is widespread [22].

The objective of this study is to find the frequency of fungal infections in patients in whom symptoms of infection are nonspecific; there is often a delay in proper diagnosis and treatment. The histological diagnosis will be confirmed by PAS stain, taken as gold standard thus identifying the most common infectious fungal organisms in our patients.

### METHODS

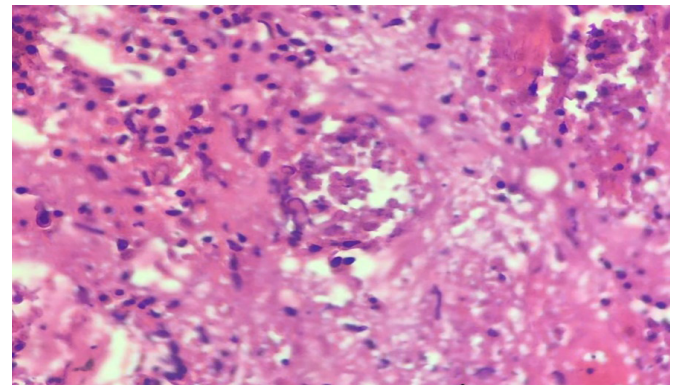
This was a retrospective descriptive study which was conducted at the histopathology department, of Kind Edward Medical University. The duration of the study was six months from November 2020 to May 2021. 5987 is the total number of specimens submitted to the pathology department within six months. 1295 were the biopsies with chronic nonspecific inflammation. 34 biopsies were diagnosed with superadded fungal infection. These patients were those who presented to outdoor, general surgery, plastic surgery, maxillofacial, burn, eye unit and ICU. After history, examination and baseline investigations biopsy was done with informed consent. Biopsies were submitted to the pathology department and after processing slides were prepared. Hematoxyllin and eosin stains were done, and for further confirmation and categorization periodic acid-Schiff stain was performed. Data was analyzed using SPSS version 20.

### RESULTS

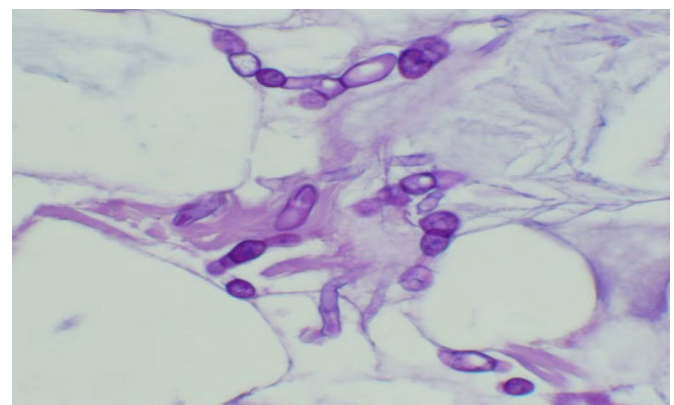
In our study, out of 34 patients, 18 (52.9%) were male and 16 (47.1%) were female (**Table 1**). The biopsies were taken from different organs and parts of the body which included the head and neck, lungs, cheeks, maxilla, palate, sinus, leg, foot, nasalcavities and necrotic patch on the extremities. Mucormycosis turned out to be the most common fungal infection in Mayo Hospital with a frequency of 23 out of 34(67.6%) (**Fig. 1**). After Mucormycosis, the second most frequent fungal infection reported to candidiasis with a frequency of 5 out of 34 (14.7%), and then aspergillosis with the frequency of 4 (11.8%) (**Fig. 2**). 2 out of 34 (5.8%) have concurrent two fungal infections (mucormycosis with candida and mucormycosis with aspergillosis) (**Table 1**).

**Table 1:** Percentage of most common fungal infections in patients presenting to the pathology department at KEMU.

Fungal Infections	Frequency	%
Aspergillosis	4	11.8
Candidiasis	5	14.7
Candidiasis and Mucormycosis	1	2.9
Mucormycosis	23	67.6
Mucormycosis and Aspergillosis	1	2.9
Total	34	100.0



**Fig. (1):** H&E stain at 40x showing hyphae of Mucormycosis (picture taken at pathology department).



**Fig. (2):** PAS stain at 40x showing spectated hyphae of Aspergillosis.

## DISCUSSION

Mucormycosis has been reported as the most dangerous and frequent fungal infection. Mucormycosis, associated with high morbidity and mortality, is an angio-invasive fungal infection. Sometimes, diagnosis is delayed, and illness continues to develop rapidly.

In patients with diabetes mellitus, the rhino-cerebral type of mucormycosis is most commonly seen, whereas pulmonary mucormycosis is most commonly Pakistan's reported data documenting rates of fungal infections in general and particular populations has been checked and used where appropriate. Estimates, in the LIFE approach, were made for the entire population or particular populations at risk. An estimated 3,280,549 (1.78%) of the 184,500,000 people in Pakistan are affected by a severe fungal infection, omitting all cutaneous infections, oral candidiasis and allergic fungal sinusitis, which we were unable to estimate. Seen in patients with haematological malignancy [23].

Candidemia (21/100,000) and mucormycosis (14/100,000) rates are estimated to be very high compared to other countries and are based on data from India. Owing to the high TB burden, chronic pulmonary aspergillosis rates are estimated to be high (39/100,000). The estimate for invasive aspergillosis was around 5.9/100,000. Fungal keratitis, with an approximate prevalence of 44/100,000, is also problematic in Pakistan. Pakistan is likely to have a high rate of many life-threatening or visually-threatening fungal infections. [24].

According to Severe Fungal Infections in Pakistan 2017, Candidemia mortality rate ranges from 23 to 52% in Karachi studies and 24% in a neonatal intensive care unit (ICU) (Patients with upper gastrointestinal disease and prolonged ICU stay have a higher proportion of intra-abdominal candidiasis), with about 25,830 cases of mucormycosis from Pakistan using a prevalence of 0.14/1000 population and 38% mortality, yearly, 2,821,440 women of reproductive age suffer from recurrent the burden of reproductive tract infection in urban women in Pakistan reports vaginal candidiasis as the second most common genital infection, with a prevalence of 7–12% [25].

Study limitations are outsourcing of samples for diagnosis, so we lose a lot of data regarding the actual load of fungal infection prevalent at our setup.

## CONCLUSION

It is concluded that the most frequent fungal infection in Mayo Hospital is reported to be Mucormycosis, keeping the PAS stain as the gold standard, with a frequency of 23 out of 34, and a percentage of 67.6%. Rest important and

common fungal infections were reported to be candidiasis, aspergillosis and combinations of mucormycosis and candidiasis, and mucormycosis and aspergillosis.

## ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Review Board of King Edward Medical University (KEMU), Lahore (REF letter No. 863/RC/KEMU, Dated: 14/11/2020). All procedures performed in studies involving human participants were following the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

## CONSENT FOR PUBLICATION

Written informed consent was taken from the participants.

## AVAILABILITY OF DATA

All data in hard and soft form is available.

## FUNDING

Declared none.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

Declared none.

## AUTHORS' CONTRIBUTION

Topic was decided and H&E stain diagnosis were made by Dr. Safeena Sarfraz.

Collection of data it's compiling and material collection was done by Myra Shahid.

Reporting of PAS stain was done by Dr. Rabia Shahid (msfarooq).

Suspected cases of fungal infection were also sent by Dr. Maryam Raana and Dr. Quratul Ain.

Statistical data was helped by Dr. Fizza Jahangir.

## REFERENCES

- Richardson MD, Warnock DW. Fungal infection: diagnosis and management. John Wiley & Sons; 2012. DOI: <https://doi.org/10.1002/9781118321492>
- Segal E, Frenkel M. Dermatophyte infections in environmental contexts. *Res Microbiol* 2015; 166(7): 564-9. DOI: <https://doi.org/10.1016/j.resmic.2014.12.007> PMID: 25634072
- Denning DW, Chakrabarti A. Pulmonary and sinus fungal diseases in non-immunocompromised patients. *Lancet Infect Dis* 2017; 17(11): e357-66. DOI: [https://doi.org/10.1016/s1473-3099\(17\)30309-2](https://doi.org/10.1016/s1473-3099(17)30309-2) PMID: 28774699
- Jeelani S, Ahmed QM, Lanker AM, Hassan I, Jeelani N, Fazili T. Histopathological examination of nail clippings using PAS staining (HPE-PAS): gold standard in diagnosis of onychomycosis. *Mycoses* 2015; 58(1): 27-32. DOI: <https://doi.org/10.1111/myc.12251> PMID: 25346218



5. Sanguinetti M, Posteraro B, Beigelman-Aubry C, Lamoth F, Dunet V, Slavin M, *et al.* Diagnosis and treatment of invasive fungal infections: looking ahead. *J Antimicrob Chemother* 2019; 74(Suppl 2): ii27-ii37. DOI: <https://doi.org/10.1093/jac/dkz041> PMID: 31222314
6. Enoch DA, Yang H, Aliyu SH, Micallef C. The changing epidemiology of invasive fungal infections. *Methods Mol Biol* 2017;1508:17-65. DOI: [https://doi.org/10.1007/978-1-4939-6515-1\\_2](https://doi.org/10.1007/978-1-4939-6515-1_2) PMID: 27837497
7. Jabeen K, Farooqi J, Mirza S, Denning D, Zafar A. Serious fungal infections in Pakistan. *Eur J Clin Microbiol Infect Dis* 2017; 36(6): 949-56. DOI: <https://doi.org/10.1007/s10096-017-2919-6> PMID: 28161741
8. Moubasher AH, Abdel-Sater MA, Soliman Z. Incidence and biodiversity of yeasts, dermatophytes and non-dermatophytes in superficial skin infections in Assiut, Egypt. *J Mycol Med* 2017; 27(2): 166-79. DOI: <https://doi.org/10.1016/j.mycmed.2017.01.005> PMID: 28188051
9. Haroon F, Iqbal Z, Pervaiz K, Khalid AN. Incidence of fungal infection of freshwater ornamental fish in Pakistan. *Int J Agri Biol* 2014; 16(2): 411-5.
10. Verma N, Singh S, Taneja S, Duseja A, Singh V, Dhiman RK, *et al.* Invasive fungal infections amongst patients with acute-on-chronic liver failure at highrisk for fungal infections. *Liver Int* 2019; 39(3): 503-13. DOI: <https://doi.org/10.1111/liv.13981> PMID: 30276951
11. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, *et al.* Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *The Lancet Infect Dis* 2019; 19(12): e405-21. DOI: [https://doi.org/10.1016/s1473-3099\(19\)30312-3](https://doi.org/10.1016/s1473-3099(19)30312-3) PMID: 31699664
12. Lecerf P, Abdy S, Vollono L, Pastushenko I, Richert B, André J. Direct examination, histopathology and fungal culture for the diagnosis of onychomycosis: a retrospective, comparative study on 2245 specimens. *Mycoses* 2021; 64(2): 187-93. DOI: <https://doi.org/10.1111/myc.13201> PMID: 33075162
13. Khan MI, Kashif M, Iqbal J, Aslam M, Waseem H, Mubbashir R, *et al.* Depression in patients with fungal infection in tertiary care hospital. *J Pak Associ Dermatol* 2019; 29(4): 402-8.
14. Leclair LW, Hogan DA. Mixed bacterial-fungal infections in the CF respiratory tract. *Med Mycol* 2010; 48 Suppl 1: S125-32. DOI: <https://doi.org/10.3109/13693786.2010.521522> PMID: 21067324
15. Kovachev SM, Vatcheva-Dobrevska RS. Local probiotic therapy for vaginal *Candida albicans* infections. *Probiotics Antimicrob Proteins* 2015; 7(1): 38-44. DOI: <https://doi.org/10.1007/s12602-014-9176-0> PMID: 25362524
16. Stamatiades GA, Ioannou P, Petrikkos G, Tsioutis C. Fungal infections in patients with inflammatory bowel disease: A systematic review. *Mycoses* 2018; 61(6): 366-76. DOI: <https://doi.org/10.1111/myc.12753> PMID: 29453860
17. Jabeen K, Irfan M. Challenges in the diagnosis and treatment of pulmonary fungal infections in Pakistan. *Pak J Chest Med* 2023; 29(3): 80-1.
18. Ullmann AJ, Akova M, Herbrecht R, Viscoli C, Arendrup MC, Arikan-Akdagli SE, *et al.* ESCMID\* guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). *Clin Microbiol Infect* 2012; 18 Suppl 7: 53-67. DOI: <https://doi.org/10.1111/1469-0691.12041> PMID: 23137137
19. Hope WW, Castagnola E, Groll AH, Roilides E, Akova M, Arendrup MC, *et al.* ESCMID\* guideline for the diagnosis and management of *Candida* diseases 2012: prevention and management of invasive infections in neonates and children caused by *Candida* spp. *Clin Microbiol Infect* 2012; 18 Suppl 7: 38-52. DOI: <https://doi.org/10.1111/1469-0691.12040> PMID: 23137136
20. Berkovits C, Tóth A, Szenzenstein J, Deák T, Urbán E, Gácsér A, *et al.* Analysis of oral yeast microflora in patients with oral squamous cell carcinoma. *Springerplus* 2016; 5(1): 1257. DOI: <https://doi.org/10.1186/s40064-016-2926-6> PMID: 27536540
21. Theocharidou E, Agarwal B, Jeffrey G, Jalan R, Harrison D, Burroughs AK, *et al.* Early invasive fungal infections and colonization in patients with cirrhosis admitted to the intensive care unit. *Clinical Microbiology and Infection*. 2016; 22(2): 189-e1. DOI: <https://doi.org/10.1016/j.cmi.2015.10.020> PMID: 26551838
22. Dubey A, Patwardhan RV, Samph S, Santosh V, Kolluri S, Nanda A. Intracranial fungal granuloma: analysis of 40 patients and review of the literature. *Surg Neurol* 2005; 63(3): 254-60. DOI: <https://doi.org/10.1016/j.surneu.2004.04.020> PMID: 15734518
23. Mushtaq K, Khan Z, Aziz M, Alyousif ZA, Siddiqui N, Khan MA, *et al.* Trends and outcomes of fungal infections in hospitalized patients of inflammatory bowel disease: a nationwide analysis. *Transl Gastroenterol Hepatol* 2020; 5: 35. DOI: <https://doi.org/10.21037/tgh.2019.10.14> PMID: 32632386
24. Jung MY, Shim JH, Lee JH, Lee JH, Yang JM, Lee DY, *et al.* Comparison of diagnostic methods for onychomycosis, and proposal of a diagnostic algorithm. *Clin Exp Dermatol* 2015; 40(5): 479-84. DOI: <https://doi.org/10.1111/ced.12593> PMID: 25683452
25. Jabeen K, Khan M, Umar S, Shaheen N, Farooqi J. Spectrum of fungal pathogens in burn wound specimens: data from a tertiary care Hospital Laboratory in Pakistan. *J Burn Care Res* 2021; 42(2): 241-4. DOI: <https://doi.org/10.1093/jbcr/iraa148> PMID: 32844184